

# Wound Healing Activity of Herbal Ointment Containing the Extracts of *Hibiscus rosa-sinensis* Flowers and *Curcuma longa* Rhizomes

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## ABSTRACT

**Objectives:** To evaluate wound healing activity of herbal ointment containing the extracts of *Hibiscus rosa-sinensis* flowers and *Curcuma longa* rhizomes. **Methodology:** *H. rosa-sinensis* flowers and *C. longa* rhizomes were extracted using 95% ethanol. *H. rosa-sinensis* and *C. longa* ointment were prepared using paraffin wax, lanolin and petroleum jelly. Ointment formulation was applied once daily on the excision wound made on Sprague Dawley rats for 20 days. **Results:** On day 20, the treatment group showed a significant increase in wound contraction which was  $93.52 \pm 1.38\%$  when compared to control ( $87.62 \pm 1.48\%$ ). **Conclusion:** Herbal ointment exhibited significant wound healing activity in rat excision wound model. **Key words:** *Hibiscus rosa-sinensis*, *Curcuma longa*, Wound healing.

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## INTRODUCTION

Herbs are a source of drug for a millennium years. From ancient times, man has used a wide range part of plants for the treatment as well as prevention of various ailments. Lately, there has been a great interest of scientific community in exploring the pharmacological activities of medicinal plants including the wound healing activity to establish the claims made about them in folklore medicines.<sup>1</sup>

Wounds can be described as cut or breaks in continuity of any tissue which are caused by physical, chemical, microbial, immunological or thermal insult to the tissue.<sup>2</sup> Wound healing is an action involves in repairing that follows injury to the skin and other soft tissues. Proper wound healing activity act as an important role in the restoration of disrupted anatomical continuity as well as disturbed functional status of skin. The aim of wound management is to heal the wound in a time as short as possible; at the same time, to minimize the pain, discomfort and chance of forming scars.

*Hibiscus rosa-sinensis* known informally as Chinese hibiscus, China rose and shoeblack plant. Flowers were reported to be effective in hypertension treatment and possessed a good antifertility effect.<sup>3</sup> Furthermore, hibiscus acts as antioxidant, expectorant with mild laxative and diuretic effect. It was also found to decrease cholesterol levels. The antioxidant, anticancer and anti-inflammatory activity of *H. rosa-sinensis* was also reported.<sup>4</sup>

*Curcuma longa* is known as kunyit in Malaysia. Aggarwal *et al.* (2013) have investigated on anti-inflammatory and anticancer activities of curcumin.<sup>5</sup>

Furthermore, the antimicrobial, antioxidant, anti-mutagenic, antidiabetic and hepatoprotective activity had been reported.<sup>6</sup>

Many studies had reported the wound healing effect of *H. rosa-sinensis* and *C. longa* and supported their respective traditional usage.<sup>7-12</sup> However, up to date there is no research to provide evidence on wound healing properties of extract combination of *H. rosa-sinensis* and *C. longa*. Hence, this research is planned to investigate wound healing activity of combination of herbal ointment containing the extracts of *Hibiscus rosa-sinensis* flowers and *Curcuma longa* rhizomes.

## MATERIALS AND METHODS

### Extraction of Plant Materials

*H. rosa-sinensis* flowers were collected in AIMST University, Kedah Darul Aman, Malaysia. The flowers petals were separated from the calyx, anther and stigma. Flowers petals were washed, wiped dry and further dried in hot air oven at 45°C. Then, the dried flower petals were blended into fine powder. The powdered flowers were extracted by maceration using 95% ethanol as the solvent for 48 hr and repeated 3 times. Later, filtrate was collected and evaporated on water bath at 40°C till a semi-solid extract was obtained. The percentage yield of the ethanolic extract of flowers of *H. rosa-sinensis* is 8.95 g w/v.

*C. longa* was purchased from Bedong local market. Rhizomes of *C. longa* were washed, wiped, dried and blended into smaller pieces by using blender. Then,

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the blended pieces were then dried in hot air oven at 45°C. Then, the dried rhizomes were blended into fine powder. The powdered rhizomes were extracted by maceration using 95% ethanol as the solvent for 48 hr and repeated 3 times. Later, filtrate was collected and evaporated on water bath at 40°C till a semi-solid extract was obtained. The percentage yield of the ethanolic extract of rhizomes of *C. longa* is 15.26 w/v.

### Preparation of ointment

Paraffin wax was placed in evaporating dish and melted on water bath. After melting of paraffin wax, lanolin (1.5 g), cetostearyl alcohol (1.5 g), paraffin wax (1.5 g) and petroleum jelly (25.5 g) were added and mixed homogeneously. Thereafter, *H. rosa-sinensis* (100 mg) and *C. longa* (100 mg) extract were weighed and mixed with ointment base by using mortar and pestle.

### Animal

Healthy, male Spring dawley rats weight  $180 \pm 20$  g were used for the experiment. The animal were kept in clean and dry cages and maintained with 12 h-light-12 h dark cycle. Rats were fed with rat pellet and water *ad libitum*. The experiment protocol was approved by the AIMST University Human and Animal Ethics Committee (Approval number: AUHAEC/ FOP/ 2019/ 04).

### Excision Wound Creation

The animals were anesthetized by using diethyl ether. After a few seconds, the dorsal fur of animals was removed, the anticipated area (~500 mm<sup>2</sup>) of wound to be created was outlined on the back of animals. Then, the wound was created by cutting away full thickness of skin along the markings area and (5 mm diameter) and the wound was left undressed to open environment. Each rat was placed in separated cage after the wound creation.<sup>13</sup>

### Wound Healing Activity

After wound creation, the animals were divided into 3 groups of five each. The animals of group I served as wound control group which were left untreated, group II as standard drug treated group which were applied with povidone-iodine solution while animals of group III were test group and applied with herbal ointment. The ointment was topically applied to the animals once a day for a period of 20 days, starting from second days of the wound creation.

### Measure of Wound Contraction

Wound contraction was examined by measuring the wound area on the alternate days. Transparent paper was used to trace the excision wound margin and the area measured by using graph paper.<sup>14</sup> The changes in wound area was expressed in mm<sup>2</sup>. Wound contraction was expressed as percentage of original wound reduction and calculated using the formula below.

$$\% \text{ of wound contraction} = \frac{\text{Initial wound size} - \text{Specific day wound size}}{\text{Initial wound size}} \times 100$$

### Statistical Analysis

Data were expressed as mean  $\pm$  standard error of mean (SEM) for five animals per group. Statistical analysis was made using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. *P*-value < 0.05 was considered to be statistically significance.

## RESULTS

The measurements of the wounds treated with povidone-iodine solution and herbal ointment are presented in Table 1. The excision wound rats applied with the herbal ointment and povidone-iodine showed

a significant wound healing activity when compared with that of control animals on day 8 onwards. Herbal ointment showed significant percentage closer activity from day 4 of the experiment and povidone-iodine solution significant percentage closer activity from day 8 of the experiment onwards when compared with that of that of control (Figure 1).

## DISCUSSION

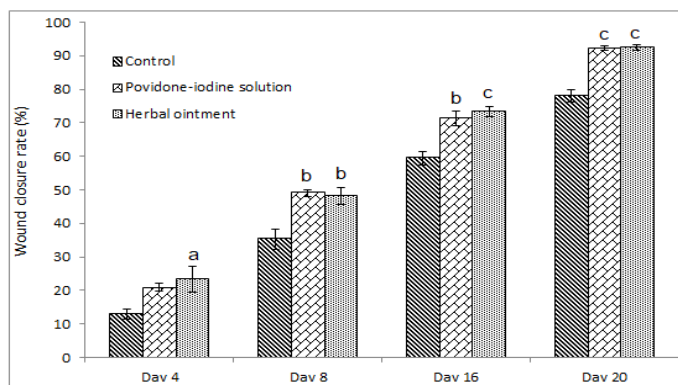
Wound healing is defined as restoration of damage tissue to its original state which is initiated by stimulus of tissue injuries.<sup>13</sup> Connective tissue repair process involves four stages which are coagulation to prevent loss of blood, inflammation and removal of foreign substance from skin (debridement), epithelial repair and lastly remodeling of tissue and deposition of collagen. The agents having properties to accelerate these stages are known as promoter of wound healing.<sup>14</sup>

Treatment group showed significant increase in wound contraction when compared to control on 8<sup>th</sup> day onwards. Wound healing activity of *H. rosa-sinensis* and *C. longa* herbal ointment might be due to antioxidant potential of both this plant.<sup>15,16</sup> Wound healing process consists of cell proliferation, suppression of inflammation and collagen contraction and these can be delayed by antioxidants. During inflammation, neutrophils and cytokines produce reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) which are acting as free radicals that exacerbate damage and inhibit tissue remodelling.<sup>17</sup> ROS can cause inactivation of enzymes, breakage of DNA as well as lipid peroxidation which will retard optimum wound healing process. In this case, antioxidants are useful to diminish the damage caused by oxidative stress thus enhance the healing process. The redox properties of antioxidant enable them to act as reducing agents, hydroxyl radicals and superoxide radical scavengers.

**Table 1: Wound healing activity of herabal formulation.**

Days after infliction of wound	Wound area (mm <sup>2</sup> )		
	Control	Povidone-iodine	Herbal ointment
2	521.20 $\pm$ 10.84	519.20 $\pm$ 10.80	525.60 $\pm$ 11.78
4	453.80 $\pm$ 13.25	411.60 $\pm$ 13.71	401.80 $\pm$ 14.66
8	335.8 $\pm$ 9.07	264.80 $\pm$ 9.57**	272.60 $\pm$ 12.52**
16	211.40 $\pm$ 11.77	150.20 $\pm$ 12.71**	140.20 $\pm$ 8.97**
20	114.60 $\pm$ 9.47	40.60 $\pm$ 3.54***	39.80 $\pm$ 5.21***

The values are given as mean  $\pm$  SEM (*n* = 5). \*\**p* < 0.01 and \*\*\**p* < 0.001 compared with controls (One-way ANOVA followed by Tukey's *post hoc* test)



**Figure 1: Wound healing activity of herbal ointment as comparison with positive and negative control.**

The values are given as mean  $\pm$  SEM (*n* = 5). <sup>a</sup>*p* < 0.05; <sup>b</sup>*p* < 0.01 and <sup>c</sup>*p* < 0.001 compared with controls (One-way ANOVA followed by Tukey's *post-hoc* test)

Therefore, the better wound healing effect of treatment group may be due to the antioxidant properties in both *H. rosa-sinensis* and *C. longa*.

Under usual physiological condition, inflammatory phase is the vital stage in wound healing process to eliminate the necrosis tissue debris as well as micro-organism. Nevertheless, prolonged inflammation will disturb or delay healing process severely and lead to increased fibrosis and scarring. Many research studies have proved the significant anti-inflammatory properties of *C. longa*. Wound that treated with *C. longa* containing curcumin showed a higher number of macrophage, neutrophils and fibroblast cells than the untreated wound. These infiltrating cells producing a variety of potential growth factors which is necessary for modulation of biological processes during wound healing. For example, transforming growth factor-beta (TGF- $\beta$ ) and epidermal growth factor (ECG) are important to stimulate the synthesis of fibronectin (FN) as well as collagen and hence elevate granulation tissue formation.<sup>18</sup> Hence, this anti-inflammatory effect may contribute to the wound healing acceleration in treatment group.

Gandhi et al. has reported the individual wound healing activity of *H. rosa-sinensis* to be 78.26% for 5% w/w extract on 12<sup>th</sup> day.<sup>19</sup> The comparison of this study taking into account the previous study, the individual extract (78.26%) wound healing activity was weaker than combination herbal ointment (93.13%). Thus, it may be concluded that combination of extract shows synergistic effect in promoting wound healing.

## CONCLUSION

Herbal ointment showed showed a significant wound healing activity on excision wounds in rats.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ABBREVIATIONS

**H. Rosa sinensis:** *Hibiscus Rosa sinensis*; **C. Longa:** *Curcuma longa*; **TGF- $\beta$ :** Transforming growth factor-beta; **ECG:** Epidermal growth factor; **FN:** fibronectin; **RNS:** Reactive nitrogen species; **ROS:** Reactive oxygen species.

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## SUMMARY

- This research was anticipated to determine the wound healing activity of herbal ointment containing the extracts of *Hibiscus rosa-sinensis* flowers and *Curcuma longa* rhizomes.
- This current research showed that the combination of *H. rosa-sinensis* and *C. longa* ointment could be used as natural wound healing agent.

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