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Preliminary evaluation: The effects of *Aloe ferox Miller* and *Aloe arborescens Miller* on wound healing

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ABSTRACT

Aim of the study: Genus Aloe has been traditionally utilized for medicinal purpose for decades. Compared with *Aloe vera gel*, the qualitative assessment for the therapeutic effects of the other two Aloe species, *Aloe ferox Miller* and *Aloe arborescens Miller*, for their topical wound healing was less addressed. Therefore, the aim of present study is to provide the positive evidence for *Aloe ferox Miller* and *Aloe arborescens Miller* supporting their therapeutic properties for topical treatment of skin wounds.

Materials and methods: Two types of the whole-leaf juice prepared from either *Aloe ferox Miller* or *Aloe arborescens Miller* were used in this study. Incision wound healing was investigated using both the rat and rabbit model. The wound closure rate with and without the topical administration of the whole-leaf juice were monitored. The changes in wound characteristics were traced and wound severity was scored on different days. The anti-microorganism actions of each whole-leaf juice preparation were evaluated by measuring their inhibition growth effects on four bacterial strains and three fungal spores. The toxic influence owing to topical application of Aloe whole-leaf juice on intact and damaged skin was also assessed.

Results and Conclusions: Our results indicated that the two types of whole-leaf juice preparations exhibit the therapeutic properties, including facilitation of the healing process, selective inhibition of the microbial growth and zero side-effect on the skin, during the observation period. It is concluded that both of Aloe whole-leaf juice preparations have the positive potential for skin medicinal application.

1. Introduction

Genus Aloe plant has been traditionally applied for the medicinal practice over thousands of years in many cultures of the world. There are at least four Aloe species that are reported having the therapeutic effects, namely, *Aloe barbadensis Miller* (syn. *Aloe vera*; Liliaceae) (Chandana et al., 2007), *Aloe ferox* (syn. *Cape Aloe*; Liliaceae) (Zahn et al., 2007), *Aloe arborescens* (syn. *Candelabra Aloe*; Liliaceae) (Morita et al., 2007) and *Aloe perryi baker* (syn. *Perry's Aloe*; Liliaceae) (Eshun and He, 2004), among which Aloe *vera* is the most widely studied species so far for its clinical effectiveness against a variety of skin disorders including burns and wounds (Chithra et al., 1998b; Vogler and Ernst, 1999; Biswas and Mukherjee, 2003). The reported positive influence of *Aloe vera* on skin wound repair, for example, anti-inflammation, antimicrobial, immunomodulation and hematopoiesis stimulation and absorbent quality, have been attributed to its diverse constituents, in particular, the polysaccharides (Duansak et al., 2003; Nia et al., 2004; Talmadge et al., 2004). Apart from polysaccharides, miscellaneous bioactive constituents have been identified from the leaves and roots of Aloe plant. These Aloe compounds belong to different classes such as alkaloids, anthraquinones, saccharides, enzymes, amino acids, inorganic mineral, etc. (Vogler and Ernst, 1999; Eshun and He, 2004). In regards to the healing properties, many researches have demonstrated that the mucilaginous polysaccharides contained in the clear pulp of Aloe leaf are the major ingredient responsible for the healing. However, new evidence has shown that emodin, one of the derivatives of anthraquinones produced by superficial pericyclic cells, is also capable of promoting the repair of rats' excisional wounds via stimulating tissue regeneration (Eshun and He, 2004; Tang et al., 2007). This is a supporting evidence to the claim that the healing function of Aloe plant is essentially a result of the synergistic mode of action of many bioactive compounds, rather than one single "magic bullet" (Dagne et al., 2000). In light of this hypothesis, we thought it worthwhile to examine whether a whole-leaf preparation which is a juice-like product made either from *Aloe ferox Miller*

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or *Aloe arborescens Miller* has an effect on acute incisional wound healing.

Aloe arborescens Miller is widely cultivated and used in Japan for its therapeutic and cosmetic purpose. However, the preparation of this Aloe species is more preferable to use the whole-leaf rather than the isolated gel (Reynolds and Dweck, 1999). *Aloe ferox Miller* is widespread in the Eastern Cape province of South Africa. This plant is traditionally used for the treatment of various diseases such as skin cancer, burns, eczema, psoriasis and so on (Loots et al., 2007). Most of the claims regarding wound healing properties of *Aloe ferox Miller* and *Aloe arborescens Miller* are based on their historical use. The controlled scientific investigation into the healing of skin injury using *Aloe ferox Miller* and *Aloe arborescens Miller* are few, compared to *Aloe vera* (Vogler and Ernst, 1999). The reason for this may be due to their limited commercial interest or the cultivation constrains (Dagne et al., 2000; Loots et al., 2007).

Phytochemical characteristics of two whole-leaf juice preparations using *Aloe ferox Miller* and *Aloe arborescens Miller* were investigated in our previous studies. The presence of aloin, Aloe-emodin, polysaccharides, mannose and acemannan were confirmed in each whole-leaf juice preparation (Jia and Kong, 1989; Jia and Gao, 1993). The polysaccharide fractions in each whole-leaf juice preparation had a molecular weight between 150 and 1000 kDa. The major sugar residue in the polysaccharide fractions is largely mannose (Jia and Gao, 1993).

Wound healing is a complex process involving three distinct and overlapping events: (1) inflammation, (2) new tissue formation and (3) maturation (McNees, 2006). Wound infection is likely the most common reason for poor wound healing. According to Centers for Disease Control and Prevention (CDC), USA, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* are the most frequently reported pathogens associated with surgical infection. In addition, fungi can also be responsible for the superficial infections of the skin, and can become an important cause of burn-associated infection. Consequently, the efficiency in eradiation of the potential pathogenic microorganisms after injury not only determined the healing speed, but also played an essential role in controlling the surgical morbidity and mortality. Moreover, dermal toxicity and relative skin irritancy in response to the topical treatment may play a role to delay or impair the wound healing. Hence it is necessary to evaluate the safety issue associated with the topical application of whole-leaf juice for skin repair.

In an attempt to understand the process of wound healing in normal human patient and relate the Aloe activity to the healing effects on acute skin wounds, an animal model will be a better choice than the *in vitro* cell culture system in a sense that to provide a replicable complex biological environment of a wound and to limit variations in temperature, pH, oxygen and nutrient supply, etc., which usually encountered *in vitro* (Myers and Gould, 2008). We have investigated the effect of the topically applied whole-leaf juice formulations on impaired incisional and excisional wounds using both the rats and rabbit models. Rats are the most common animal species used for the study of skin wound healing, mainly because of their availability and low cost (Greenhalgh, 2005). However, rats skin is significantly different from human skin in respect of too loose to adhere to the underlying structure, resulting in an accelerated cutanenous wound healing in rats. The predominant form of wound healing in rats is by contraction rather than epithelialization which is found for humans (Dorsett-Martin, 2004; Greenhalgh, 2005). Due to such limitations of rat model, a dermal wound in rabbit model was also considered in this study. In rabbit model, a larger skin area can be provided; thereby a granulation tissue which is essential for healing in humans can be obtained. The healing of the wound in rabbits was judged by the postoperative re-epithelialization rate of the wounds (Myers and Gould, 2008).

In this work, preliminary attempts to test the stimulation effects of *Aloe ferox Miller* and *Aloe arborescens Miller* on wound healing were performed by (1) tracing the changes in wound status over a given period of time, (2) scoring the wound severity at progressive time points, (3) evaluating the inhibition of bacterial growth, and (4) assessing the dermal toxicity. Two types of whole-leaf juice, which were prepared from individual Aloe species of interest, were employed throughout the study. An incision wound model was created in our study using both the rat and rabbit, along which the healing by first intention and second intention were both considered. However, due to the limited number of animals for the test, here leave a regret of being incapable to conduct the evaluation of healing rate for each Aloe preparation in parallel in both the rat and rabbit model.

The goal of this study was to build a direct correlation between the accelerated wound healing and the topical application of Aloe preparations. Because the chemical components in each Aloe preparation were not significantly different as indicated from previous results, neither the comparison of the medicinal effectiveness between *Aloe ferox Miller* and *Aloe arborescens Miller*, nor the investigation of the dose–reaction relationship was included at this early stage of the study, although they are key points in biological activities studies using animal models. Once the positive role of Aloe preparation in wound healing was established, the efforts in the future will give to the dose–response relationship in animal studies, upon which will guide us to refine current Aloe formulations for an enhanced therapeutic effect.

2. Materials and methods

2.1. Preparation of the whole-leaf juice

The Aloe leaf samples were harvested from an *Aloe ferox Miller* (*Cape Aloe*, Liliaceae) plant and an *Aloe arborescens Miller* (Liliaceae) plant at 2 years of age. Mature leaf samples of *Aloe arborescens Miller* were collected from Industrial Aloe Plantation, Changle, Fujian, China, whereas *Aloe ferox Miller* leaf samples were collected from Tropical Arboretum, Nangjing, China. The fresh leafs were rinsed with distilled water. The middle section (200 g) of the entire fresh leafs with the outer cuticle was transversely cut and the chunks were grounded in a mixer followed with homogenization to give a homogeneous mass. The obtained raw Aloe product underwent coarse filtration at 8000 rpm, 4 °C for 30 min to remove the cellular fibres. Ascorbic acid (2‰) was added to the supernatant, and the obtained Aloe juice (approximately 80 ml) was sealed in a brown bottle and stored at 4 °C until use. Swabs adequately soaked in a given volume of the whole-leaf juice were applied to the test area for a maximum of 1 min.

2.2. Wound healing test

2.2.1. Experimental animals

Male Wistar rat and New Zealand white rabbits were used for this study. All animals were housed at the animal care facilities of Chinese People's Liberation Army General Hospital. All experimental protocols were performed under the approval from the Hospital Animal Care and Use Committee for animal investigations. Food pellets and water were provided ad libitum throughout the experiment. Except the agents under study, no topical or systematic therapy was given to animals.

2.2.2. Incision wounds creation and the treatment

In the rat model ($n = 5$, 200–240 g), only the whole-leaf juice of *Aloe ferox Miller* was employed for the treatment. Each rat was shaved on the back (3 cm × 5 cm) after anaesthetising with the

intraperitoneal injection of thiopentone sodium. A standardized 3-cm longitudinal incision wound was created in the shaved skin and the cutaneous muscles. The sutures were made with two stitches to secure the edges. After wound creation, the rats were randomly allocated to three groups of five rats each. In the group-I, the rats were immediately treated with topical 2 ml of the whole-leaf juice only. In the group-II, the rats were first topically treated with 2 ml of the whole-leaf juice, followed by applying 2 ml of *Staphylococcus aureus* (ATCC 25923) to the wounded area. The group-III did not obtain any treatment and was thus used as the control. Repetitive topical applications of the whole-leaf juice were carried out in the group-I and -II for each succeeding 8 h post-surgery for 2 days. The rats were sacrificed on the fourth day after injury. The progression of wound closure was examined for each group on the daily basis over a 4-day period of time. The wound severity was scored on Day-1 and -3 post-injury.

In the rabbit model ($n = 3$, 2.5 ± 0.3 kg, 4-month-old, both sex), only the whole-leaf juice of *Aloe arborescens Miller* was used for treatment. Each rabbit was anaesthetised with ketamine and then the skin of a rear leg was shaved. Under the sterilized condition, two types of incision wounds, namely the "linear-incision" and the "punch-incision", were excised on the rear leg of the rabbits. Standardized "linear-incision" wounds, 5 cm × 0.5 cm (length × depth), were induced using a surgical scalpel and closed with four stitches. The "punch-incision wounds, 1 cm × 0.3 cm (length × depth), were created using a biopsy forceps at five different spots within the shaved area. These procedures generated the wounds in both the epidermis and the dermis layers. The rabbits having "linear-incision" wounds were equally assigned to two groups, group-I and -II. Rabbits in group-I were treated topically with 3 ml of the whole-leaf juice four times per day, while those in group-II were treated with physiological saline solution and used as the control. Similarly, rabbits having the "punch-incision" wounds were equally allocated into group-III and -IV. The former group received topically 3 ml of the whole-leaf juice four times per day, the latter group was used as the control and thus only received physiological saline solution for treatment in the meantime. The healing of group-I to -IV was examined every day post-surgery for 4 days and the wound severity was scored on Day-1 and -4 post-injury.

2.2.3. Wound severity scoring

Wound healing outcomes assessed based on the standard protocols and validation systems are widely adopted in clinical practice. The resulting statistical information was provided for physicians to generate solutions (Harrison et al., 2002; Bolton et al., 2004; Grey et al., 2006). Different scoring tools have been used by researchers to assess the skin reaction toward the Aloe treatment (Dudek et al., 2000; Biswas and Mukherjee, 2003; Maenthaisong et al., 2007).

Wound severity assessment in this study was based on Bates–Jensen Wound Assessment Tool (BWAT) (McNees, 2006). Total eight item descriptors which were used to mark the wound features are summarised in Table 1. Each feature was scored on a five-point scale. A score of 5 represents the most severe outcome for a particular feature, with 1 representing the least severe. A total wound severity score can be derived by adding the individual item scores. The wound was visually examined and scored by a single observer.

2.3. Antimicrobial test

2.3.1. Preparation of bacterial culture

Bacterial strains employed in this study were *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumoniae* (ATCC 13883) and *Pseudomonas aeruginosa* (ATCC 27853). All these strains were obtained from the Microbiology Laboratory of Chinese People's Liberation Army General Hospital.

From stock, a sample of each bacterial culture was inoculated in liquid LB medium at 37 °C for 16 h till the density reach 10^9 cells/ml. Two types of Aloe-containing mediums were prepared by mixing 1 ml of each whole-leaf juice with one aliquot (1 ml) of the double strength nutrient LB medium for use. One aliquot (0.1 ml) of each bacterial inoculation was added to each Aloe-containing medium solution (2 ml), followed with incubation at 37 °C with shaking. The experiment was performed in triplicate and the mixture of sterilized distilled water with bacterial culture was used as the control. Turbidity (OD_{600}) as a measurement of the bacterial growth was recorded every 6 h onwards after mixing. Twenty-four hours later, an aliquot of the clear culture was inoculated with normal human pooled plasma and grown at 37 °C overnight. Bacterial growth in

Table 1
Item descriptors, according to the Bates–Jensen Wound Assessment Tool (BWAT), used to assess the wound features and their score scheme were summarized

Item descriptor	1	2	3	4	5
Edges	Indistinct, diffuse, no clearly visible outlines	Distinct, outline clearly visible, attached, even with wound base	Well-defined, not attached to wound base	Well-defined, not attached to base, rolled under, thickened	Well-defined, fibrotic, scarred, or hyperkeratotic
Undermining	None present	Undermining < 2 cm in any area	Undermining 2–4 cm involving < 50% wound margins	Undermining 2–4 cm involving > 50% wound margins	Undermining > 4 cm or tunneling in any area
Exudate type	None	Bloody	Serosanguineous: thin, watery, pale red/pink	Serous: thin, watery, clear	Purulent: thin or thick, opaque, tan/yellow, with or without odor
Exudate amount	None, dry wound	Scant, wound moist but no observable exudate	Small	Moderate	Large
Skin color surrounding wound	Pink or normal for ethnic group	Bright red and/or blanches to touch	White or grey pallor or hypopigmented	Dark red or purple and/or non-blanchable	Black or hyperpigmented
Peripheral tissue edema	No swelling or edema	Non-pitting edema extends < 4 cm around wound	Non-pitting edema extends > 4 cm around wound	Pitting edema extends < 4 cm around wound	Crepitus and/or pitting edema extends > 4 cm
Peripheral tissue induration	None present	Induration, < 2 cm around wound	Induration 2–4 cm extending < 50% around wound	Induration 2–4 cm extending > 50% around wound	Induration > 4 cm in any area around wound
Epithelialization	100% wound covered, surface intact	75–100% wound covered and/or epithelial tissue extends > 0.5 cm into wound bed	50–75%wound covered and/or epithelial tissue extends to 0.5 cm into wound bed	25–50% wound covered	< 25% wound covered

Each feature was scored on a five-point scale. A score of 5 represents the most severe outcome for a particular feature, with 1 representing the least severe. A total wound severity score can be derived by adding the individual item scores.

normal human pooled plasma was monitored in the same manner as that in broth medium.

2.3.2. Preparation of fungal culture and the susceptibility test

Fungi including *Candida albicans*, *Cryptococcus neoformans*, and *Trichophyton rubrum* used in this study were isolated from the clinical patients in the hospital and identified by Chinese People's Liberation Army General Hospital.

Fungal strains were grown in YM broth at 30 °C overnight, giving a final inoculum concentration of $(3–5) \times 10^4$ cfu/ml by McFarland standard. Twenty microliters of an overnight culture of each fungal strain to be tested was added to 10 ml of molten Sabouraud's Dextrose Agar (Difco) following NCCLS guidelines, mixed, and allowed to solidify in petri dishes.

Paper discs (6 mm) impregnated with 20 μl of either whole-leaf juice were placed on the surface of the seeded SD agar in triplicate tests. Plates were allowed to stand for 2 h for diffusion. Then, the plates were incubated at 35 °C for 24 h, after which the susceptibility of each organism to both of the whole-leaf juice was estimated by measuring the diameters of the inhibition zones. The susceptibility criteria were set as follows: >0.5 cm, inhibitory; ≈0.5 cm, likely inhibitory; <0.5 cm, no inhibitory.

2.4. Skin toxicity test

2.4.1. Animals for skin irritation tests

Healthy mice, Wistar rat and white Guinea pigs were used for skin irritation study. The animals were shaved on the back of the body, leaving a 4 cm × 5 cm of the nude skin (account for 20% of the total body area) for study.

2.4.1.1. Irritation to healthy skin. The shaved mice ($n = 5$, 19 ± 1 g) were left under close observation for 24 h in order to ascertain no abnormal skin responses including irritation, post-positive swelling, redness, itching, and inflammation or any other symptoms present in the shaved area. The tested mice were then equally divided into four groups. The group-I and -II were treated topically with the whole-leaf juice of *Aloe ferox Miller*, 0.5 and 1.0 ml, respectively, three times daily. The group-III and -IV received treatment with the whole-leaf juice of *Aloe arborescens Miller* in the identical manner to those in group-I/II. The systematic responses, in particular, the local skin reactions, to the topical treatment were monitored on the daily basis for successive 14 days until the mice were sacrificed.

2.4.1.2. Irritation to damaged skin. Wistar rats ($n = 10$, 220 ± 10 g) were used for skin abrasion test. Skin abrasion was made using a coarse sandblaster across over the bare skin until the presence of the noticeable tissue fluid, but not blood. The methods of grouping and dosing were the same as that for healthy skin described above.

2.4.1.3. One-dosage irritation. Shaved white Guinea pigs ($n = 10$, 280 ± 10 g) were under close observation over 24 h ensuring no abnormal skin response appeared. The pigs were equally divided into two groups and each of the groups was applied topically with 1 ml of either whole-leaf juice. The completion of the first treatment was set as time zero. Induced local dermal reactions, particularly erythema and edema, were monitored 1, 6, 24, 48 and 72 h following the first treatment.

2.4.1.4. Multiple-dosages irritation. Shaved Guinea pigs ($n = 10$, 280 ± 10 g) were grouped as described above. Each group was repeatedly treated with topical 1 ml of either whole-leaf juice once per day for successive 7 days. The Aloe administration was discontinued on the eighth day. Two variables, erythema and edema, were monitored over 10 days from the first treatment.

Table 2
The severity of skin reaction in response to local dermal irritation was assessed against two variables, erythema and edema, and graded on a four-point scale

Observation item	Symptom	Scores
Erythema	None	0
	Barely seen	1
	Visible	2
	Moderate to severe	3
	Dark red erythema with scab	4
Edema	None	0
	Barely seen	1
	Bump with clear boundary	2
	Bump up about 1 cm	3
	Bump up >1 cm, range increase	4

A larger score represents more severe reaction of the skin.

2.4.2. Allergy test

White Guinea pigs ($n = 10$, 280 ± 10 g) were shaved on the left-side back of the body over an area of 3 cm × 3 cm and were randomly and equally divided into three groups. The group-I and -II were topically applied with 0.2 ml of each whole-leaf juice preparations. Group-III was applied with 1% of 1-chloro-2,4-dinitrobenzene (0.2 ml per pig) as positive control. On the subsequent seventh and fourteenth days after the first treatment, pigs in each group received the same dose treatment in an identical manner to that in the first time. Fourteen days after the last administration, the tested pigs of each group were shaved on the right-side back of the body over a 3 cm × 3 cm. In order to compare with their left-side counterparts, the shaved skin was subject to the challenge three times in total by topically applying the whole-leaf juice at the same dose and in an identical manner to that for the left-side. Skin allergic features were marked over 3 days after the completion of the treatments.

2.4.3. Scoring system for skin irritation and allergy

Dermal toxic reactions to the skin challenge were evaluated based on the scoring system from the guideline of "Chinese Drug Pharmaco-Toxicological Study and the Technical Requirements". Detailed scoring method for skin irritation and allergy response were summarized in Tables 2 and 3, respectively. The resulting marks reflected the score of each individual symptom evaluated.

2.5. Statistical analysis

Statistical analysis was carried out using Origin 6.0 professional. Results were showed as mean ± S.D. The paired Student's *t*-test was used with a significance level of $p \leq 0.05$.

Table 3
The severity of skin allergic reaction in response to local dermal challenge was assessed against two variables, erythema and edema, and graded on a four-point scale

Observation item	Symptom	Scores
Erythema	None	0
	Minor	1
	Moderate	2
	Severe	3
	Edema plus erythema	4
Edema	None	0
	Minor, slight	1
	Moderate	2
	Severe	3

A larger score represents more severe reaction of the skin.

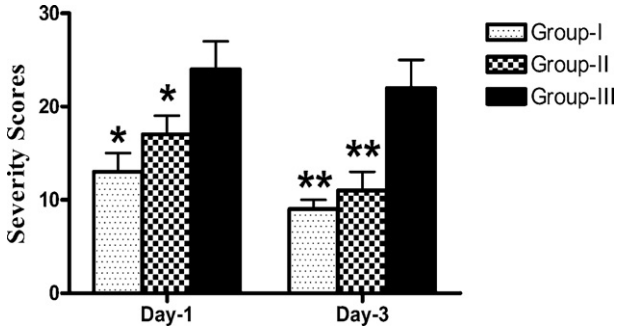

Fig. 1. Wound severity scores of group-I, -II and -III were assessed on Day-1 and -3, respectively. Significant differences ($p \leq 0.05$) in wound status were found between Aloe-treated (*Aloe ferox Miller*) groups and untreated group on Day-1 and -3. A significant improvement in wound healing was found in group-I and -II between Day-1 and -3. However, no significant difference in the healing was found in control group between Day-1 and -3. Data represent means ± S.D. ($n = 5$). * and **represent significant different at $p \leq 0.05$.

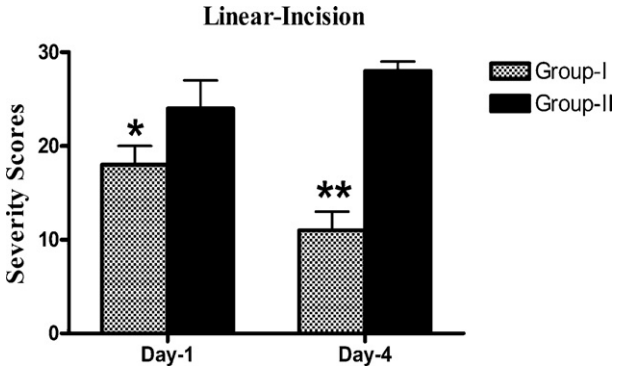

Fig. 3. Wound severity scores for "linear-incision" wounds in group-I and -II were assessed on Day-1 and -4, respectively. During the entire observation period, significant improvement ($p \leq 0.05$) in the wound status was found in Aloe-treated group-I, compared with the control group. Meanwhile, prolonging the Aloe-treatment but at the same dose give better healing outcome. Data represent means ± S.D. ($n = 3$). * and **represent significant different at $p \leq 0.05$.

3. Results

3.1. Wound healing effect

In rat model, wound severity scores of locally treated group-I and -II proved to be significantly lower than that in the control group on Day-1 and -3 ($p < 0.05$) (Fig. 1). It was found that all rats, except one in group-III, recovered from anesthesia or surgery, or both, 18–24 h after the injury. On Day-1 after injury, the incision in group-I was well approximated to each other but not completely epithelialized, meanwhile, showed no signs of inflammation. The healing of group-II, characterized by a small amount of yellow exudates and a discernable unjoined seam on the edge of the wound, was not yet complete. In group-III, a poor wound condition was observed and it was featured by bleeding and skin swelling. On Day-3 after injury, wound area in the rats of group-I and -II appeared to be almost closed, accompanied with a well-defined healing ridge. Meanwhile, the stitches in the rats of these two groups have been shed automatically. In addition, infections was not induced or deteriorated in respective group-I and -II throughout the observation period. In contrast, the rats in group-III were far from reaching a complete recovery at this time point because anastomotic seams still persistently open and the healing ridge at the wound site was hardly palpable due to the skin swelling. The statistical data of wound severity for group-I and -II did differ significantly between Day-1 and -3 (Fig. 1). Meanwhile, the large severity scores (24, Day-1; 22, Day-3) found in untreated group indicated a poor wound status and impeded healing during the observation time. Fig. 2(1–3) shows the status of the wounds 4 days after surgery. Picture was taken immediately after the rats were sacrificed. Rat 1, 2, and 3 belonged to the group-I, -II and -III, respectively.

In the rabbit model, the effects of *Aloe arborescens Miller* on the healing of "linear-incision" wounds in group-I and -II on Day-1 and -4 post-surgery were assessed and the scores were shown in Fig. 3. Data revealed that an improved wound status was obtained for rabbits in group-I, resulting from continuously topical Aloe treatments for 4 days. The scores of wound severity for Aloe-treated

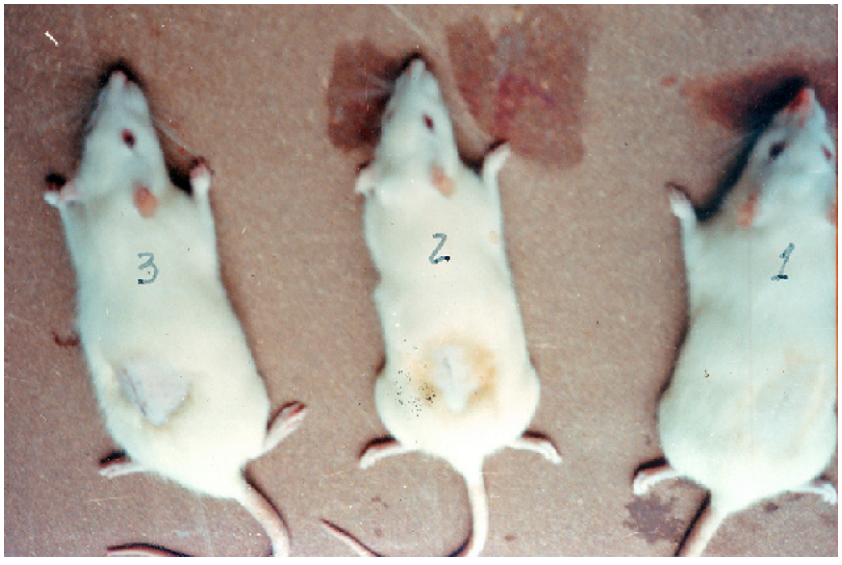

Fig. 2. In the rat model, the progression of wound healing in group-I, -II and -III were recorded. The picture was taken on Day-4 post-surgery. (1) accelerated healing was found in group-I which was topically treated with the whole-leaf juice immediately after skin injury, and received continuous treatments with the whole-leaf juice for each succeeding 8 h post-surgery for 2 days; (2) bacterial infection was inhibited in group-II which received topical application of the whole-leaf juice immediately after skin injury, following with the application of *Staphylococcus aureus* (ATCC 25923). A continuous treatments was carried out with the whole-leaf juice for each succeeding 8 h post-surgery for 2 days; (3) a relatively slow healing was observed in control group which received no treatment after injury throughout the entire time course.

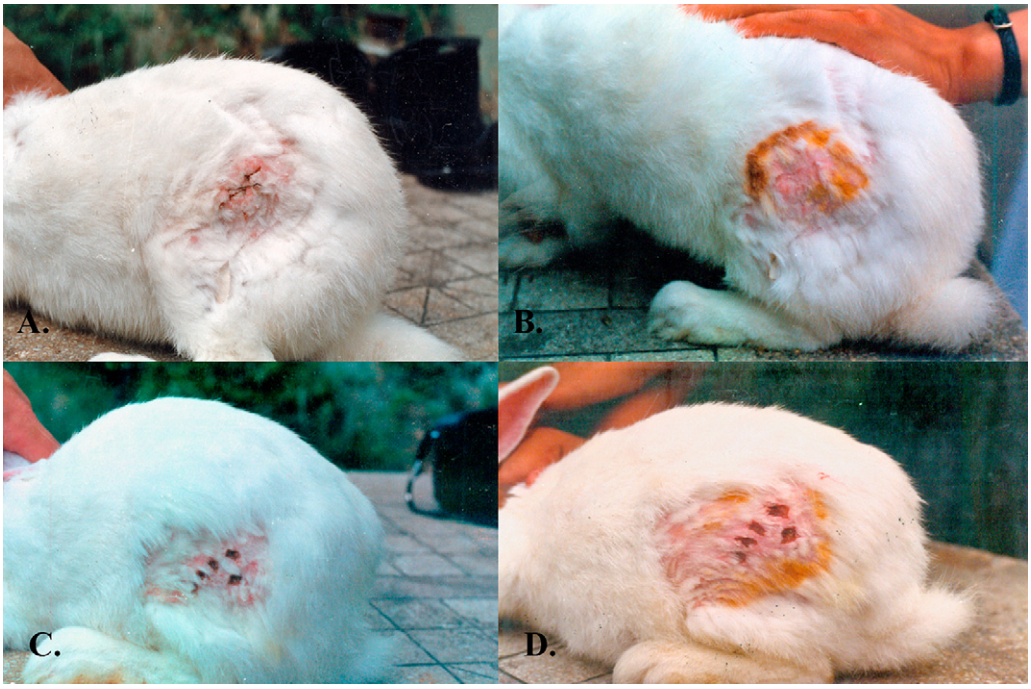

Fig. 4. In the rabbit model, the progression of wound healing for "linear-incision" and "punch-incision" wounds were recorded on the fourth day after wound creation. Original magnification: 1×. (A) A noticeable clean closure of the "linear-incision" was obtained in group-I by the continuous treatments with the whole-leaf juice four times a day. (B) Symptoms such as skin inflammation and swelling in the "linear-incision" wounded area were observed in control group which only received physiological saline solution for treatment. (C) The formation of scabs within the wounded area was observed in group-III which had "punch-incision" wound and was treated by the topical application of the whole-leaf juice four times a day. (D) Symptoms such as the skin swelling and a large volume of yellow exudate were observed in the "punch-incision" wounded area of group-IV which only received physiological saline solution for treatment. Data represent mean ± S.D. ($n = 3$).

group were remarkably lower than that for saline-treated group on Day-1 and -4. On Day-1 post-incision, a clean closure of the wound in group-I was accomplished by topical application of the whole-leaf juice in conjunction with the first intention healing. In addition, neither bloody exudate nor swelling symptoms was observed based on BWAT. Meanwhile, the presence of slight bleeding, incisional separation and skin swelling were observed in the control group. On Day-4 post-incision, Aloe-treated wound displayed a clean, well-approximated closure with a palpable healing ridge along the incision (Fig. 4A). Moreover, the wounded rabbits of group-I were staying comfortable during the treatments. However, wounded rabbits in the control group exhibited signs of delayed healing at this time point characterized by the presence of heavy pyogenesis, extensive swelling and the incisional gap (Fig. 4B). In addition, the rabbits of the control group were physically resistant to the saline solution treatments.

For "punch-incision" wound healing by second intention, the evaluation scores for the wound status in Aloe-treated group and saline-treated control group on Day-1 and -4 were shown in Fig. 5. It revealed that topical treatment with *Aloe arborescens Miller* tended to significantly reduce the wound severity with respect to that with saline treatment. Healing progressed in group-III on Day-4 with respect to Day-1 ($p < 0.05$). In Aloe-treated group-III, wounds examined on Day-1 post-injury were characterized with skin contraction and coagulation plus a dried wound surface. These healing outcomes were attributed to the topical administration of the whole-leaf juice. The formation of the scabs was further observed on the fourth day after surgery (Fig. 4C). However, an opposite situation was observed in control group. An open wound along with skin swelling and a heavy volume of exudate was observed on Day-1 after injury. Such symptoms became more severe on the fourth day as the drainage of pus and swelling persist (Fig. 4D).

3.2. Antibacterial and antifungal effects

The antibacterial effects in broth and normal human pooled plasma were shown in Table 4. After incubation for 24 h, no bacterial growth was observed for all bacterial strains incubated in both Aloe-containing mediums. In contrast, a remarkable increase in the turbidity was observed in all bacterial strains after 6 h incubation in the distilled water. This behavior indicated that both of Aloe juice had effectively inhibited the bacterial growth for four bacteria during the observation period of time.

The results of inhibitory effect of the whole-leaf juice (*Aloe ferox Miller* and *Aloe arborescens Miller*) on three fungal spores were summarized in Table 4. It showed that two fungal spores, *Candida*

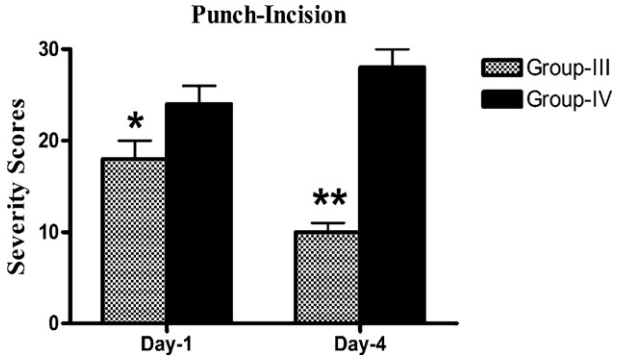

Fig. 5. Wound severity scores for "punch-incision" wounds in group-III and -IV were assessed on Day-1 and -4, respectively. During the entire observation period, significant improvement ($p \leq 0.05$) in the wound status was found in Aloe-treated group-III, compared with the control group. Meanwhile, prolonging the Aloe-treatment but at the same dose give better healing outcome. Data represent mean ± S.D. ($n = 3$). * and **represent significant different at $p \leq 0.05$.

Table 4
Results of antimicrobial effect of the whole-leaf juice, made from either *Aloe ferox Miller* (FW) or *Aloe arborescens Miller* (AW), on the bacterial strains of *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumoniae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853), and fungal spores of *Candida albicans*, *Cryptococcus neoformans* and *Trichophyton rubrum*

Medium	*Aloe*	*E. Coli*	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Candida albicans*	*Cryptococcus neoformans*	*Trichophyton rubrum*
Broth	FW	–	–	–	–	+	1.2 ± 0.05[a]	+
	AW	–	–	–	–	+	0.5 ± 0.03[a]	+
	Control	+(6)[b]	+(6)	+(6)	+(6)	+	+	+
Plasma	FW	–	–	–	–			
	AW	–	–	–	–			

The inhibitory growth was measured by monitoring the turbidity at OD_{600} after incubation at 37 °C for 24 h. +: growth; −: no growth. The susceptibility was measured by the disc diffusion method after incubation at 35 °C for 24 h. Zone diameters: >0.5 cm, inhibitory; ~0.5 cm, likely inhibitory; <0.5 cm, no inhibitory. The data represented three individual measurements and expressed as mean ± S.D. For bacteria: − = no growth, + = growth. For fungi:+ = no inhibition zone.

[a] Indicate the diameter of the inhibition zone (in cm).

[b] Indicate the time (in hour) that the turbidity observed.

Table 5
Evaluation scores on the skin irritation response of white Guinea pigs to the topical application of two aloe preparations

Observation item	Scores				
	1 (h)	6 (h)	24 (h)	48 (h)	72 (h)
Erythema formation (FW)	0	0	0	0	0
(AW)	0	0	0	0	0
Edema formation (FW)	0	0	0	0	0
(AW)	0	0	0	0	0

Observation item	Scores									
	1 (day)	2 (day)	3 (day)	4 (day)	5 (day)	6 (day)	7 (day)	8 (day)	9 (day)	10 (day)
Erythema formation (FW)	0	0	0	0	0	0	0	0	0	0
(AW)	0	0	0	0	0	0	0	0	0	0
Swell formation (FW)	0	0	0	0	0	0	0	0	0	0
(AW)	0	0	0	0	0	0	0	0	0	0

The scores for one-dosage application of either *Aloe ferox Miller* (FW) or *Aloe arborescens Miller* (AW) was obtained over a 72-h time course. The scores for multiple-dosage applications of either *Aloe ferox Miller* (FW) or *Aloe arborescens Miller* (AW) were obtained over a 10-day time course.

albicans and *Trichophyton rubrum*, exhibited none susceptibility to both Aloe-containing mediums and distilled water monitored 24 h after incubation. However, fungous spore, *Cryptococcus neoformans*, was susceptible to Aloe-containing medium, compared with the control. The growth inhibition zone displayed for *Cryptococcus neoformans* was 1.2 ± 0.05 cm for *Aloe ferox Miller* and 0.5 ± 0.03 cm for *Aloe arborescens Miller*.

3.3. Toxicity

In skin irritation test, no irritation symptoms were developed in both healthy and injured skin over a 14-day time period, regardless which one of the whole-leaf juice was applied at the given dose. The mice possessing either intact or damaged skin rapidly returned to the routine activity after topical administration of Aloe juice. On the fourteenth day, the growing hair in shaved area plus gaining body weight were observed in mice with healthy and damaged skin.

The evaluation scores of irritation response to one-dosage and multiple-dosages application of either whole-leaf juice were present in Table 5. Neither the erythema formation nor skin swelling were developed during a 72-h time period for one-dosage test and a 10-day time period for multiple-dosage test.

In the allergy test, no erythema and edema were induced in the white Guinea pigs upon the application of two Aloe juice during 3 days observation time. The evaluation scores (Table 6) marked on Day-1 following the completion of the skin challenge indicated that the application of 1-chloro-2,4-dinitrobenzene stimulated not only

Table 6
Evaluation scores on skin allergic responses of white Guinea pigs to the topical application 0.2 ml of two whole-leaf juice and 1-chloro-2,4-dinitrobenzene on Day-1

Mice no.	Scores on the formation of erythema			Scores on the formation of edema		
	Positive control	*Aloe ferox mill*	*Aloe arborescens mill*	Positive control	*Aloe ferox mill*	*Aloe arborescens mill*
1	3	0	0	2	0	0
2	3	0	0	1	0	0
3	3	0	0	2	0	0
4	3	0	0	2	0	0
5	3	0	0	2	0	0
6	3	0	0	1	0	0
7	3	0	0	2	0	0
8	3	0	0	1	0	0
9	3	0	0	2	0	0
10	3	0	0	2	0	0

the formation of severe erythema but also the moderate edema in 100% of tested white Guinea pigs. Meanwhile, intensive skin swelling was also developed within approximately 70% of tested white Guinea pigs in control group. Erythema and swelling started diminishing in the following 2 days, but not complete disappeared on the third day.

4. Discussion

It is known that timely healing is a long-lasting issue encountered by all clinicians. Nowadays, a satisfactory healing outcome, coupled with a desired healing rate, remains a challenge to wound recovery. Wound healing is known to be a complex and dynamic process which usually involves distinct phases marking the healing stages and requires multiple cell types to complete a variety of cellular activities (Chithra et al., 1998a; McNees, 2006). The major issue addressed in this study was determining the effect of two whole-leaf juice preparations on incisional wound healing and evaluating their therapeutic potential for topical application.

Data present here indicate that two of whole-leaf juices, prepared from *Aloe ferox Miller* or *Aloe arborescens Miller*, are capable to accelerate the progression of wound closure. Improved healing benefited from the Aloe treatment using either of whole-leaf juices. An increase in the rate of wound healing, characterized by a shortened period of time for fully or partially epithelialization, is marked by the better wound severity scores. Such stimulation effect of Aloe preparations took into effect in two incisional wounds which the healing underwent the first and second intention.

To date, over 130 biologically active compounds have been recognized in most of Aloe species, the relationship between various Aloe components and their wound healing effects has not yet been well elucidated. Aloe contains various carbohydrate constituents. Polysaccharides, mannose and acemannan are identified constituents of the carbohydrates presented in two Aloe preparations for this study. Polysaccharides are known to have effective property in skin wound repair. Polysaccharides fractions was capable to promote a cascade biological activities, including augmentation of reticuloendothelial function, modulation of immune responses, promotion of antiviral activity, and stimulation of hematopoiesis. Each of these biological responses has its own benefit to healing outcome (Reynolds and Dweck, 1999; Leung et al., 2004; Talmadge et al., 2004). Polysaccharides isolated from *Aloe vera gel* are largely composed of sugar mannose. Mannose binds to the certain receptors on the surface of fibroblasts, stimulating them, activating their faster growth and cell replication (East and Isacke, 2002). Davis et al. reported that mannose-6-phosphate extracted from *Aloe vera* can remarkably improve wound healing and inhibit inflammation in mice (Davis et al., 1994). Acemannan, a highly acetylated β-1,4 polymer of mannose, is known to induce the production of several inflammatory cytokines, some of which are thought to regulate wound healing (Barbul, 1990). In addition, acemannan can exert the antiviral and antitumoral activities in vivo via activation of macrophages, creation of NO and enhancement of cytotoxic T-lymphocytes (Lee et al., 2001; Choi and Chung, 2003).

Anti-inflammation is the first step in the wound healing and this effect of two Aloe preparations is believed to play a direct role in facilitating the fast healing. Topical administration of the whole-leaf juice preparations, either *Aloe arborescens Miller* or *Aloe ferox Miller*, inhibit the growth of all bacterial strains tested and are fungitoxic to *Cryptococcus neoformans* only. The obtained inhibition zone might be attributed to the greater susceptibility of *Cryptococcus neoformans* toward two whole-leaf juice preparations than to the control. In other words, *Aloe ferox Miller* might possibly be more potent in inhibiting *Cryptococcus neoformans* growth than did *Aloe arborescens Miller*. However, cautions could not be precluded because the variability in agar diffusion rates between two different Aloe preparations could also determine the inhibitory zone size in addition to the potency comparisons. Therefore, a conclusive comparison of the fungicidal activity between *Aloe ferox Miller* and *Aloe arborescens Miller* against *Cryptococcus neoformans* could not reach at this point.

Anthraquinones was allegedly a pharmacological active compound. Two derivatives of anthraquinones, aloin and Aloe-emodin, have been recognized in our Aloe samples. The claimed therapeutic applications for anthraquinones and its derivatives rely on their purgative action, anti-inflammatory activity, antiprotozoal action and antioxidant activity (Choi and Chung, 2003). *Aloe ferox Miller* is one of the most frequently used plant in South Africa for the treatment of sexually transmitted infections (Kambizi et al., 2004). Kambizi et al. found that three compounds, Aloe-emodin, aloin A and chrysophanol isolated from *Aloe ferox Miller* were active against various bacterial strains. In their results, Aloin A showed the lowest minimum inhibitory concentration (MIC) against *Staphylococcus aureus* (MIC = 62.5 mg/ml); Aloe-emodin was most effective against *Escherichia coli* (MIC = 62.5 mg/ml); while chrysophanol was more active against *Staphyloeoeeus epidermidis* (MIC = 31.25 mg/ml) (Kambizi et al., 2004). Makino et al. found that two Aloesin esters isolated from *Aloe arborescens Miller* are of the anti-inflammatory properties (Makino et al., 1974). Fujita et al. confirmed that a powder preparation of the whole-leaf of *Aloe arborescens Miller* exert its fungicidal activity against three strains of *Trichophyton mentagrophytes* by inducing morphological abnormalities in spores and hyphae (Fujita et al., 1978). Direct evidence from our results for the antifungal activity against *Candida albicans* and *Trichophyton rubrum* is not satisfactory, and this may be attributed to the presence of the active compounds in such a short amount that could not cause inhibition to them. Apart from this, Ali et al. once pointed out that a few factors, for example, the nature of solvent used to extract the Aloe components, the physical status of Aloe leaf (fresh or dry), may also influence the inhibitory efficacy (Ali et al., 1999).

The dermal toxicity was one of the issues associated with the topical application of the whole-leaf juice for wound healing. By applying 2–3 ml of the prepared whole-leaf juice to the intact or damaged skin, no signs of irritant contact were observed. In a study conducted at Institut Francais de Recherches et Essais Biologoques, *Aloe ferox Miller* leaf extract (0.5 ml) was applied to the shaved skin of white New Zealand rabbits. Their results showed that a slight erythema was developed in the damaged tissue in one of six rabbits and such symptom diminished after three days ((F) Final report on the safety, 2007; Ryan et al., 2007). A case of the side-effect associated with *Aloe ferox* leaf extract was reported, when it was instilled into the eye of white New Zealand rabbits, for the induction of minor changes in the eye. Such changes in the eyes became visible after instillation for one hour and vanished after one day ((F) Final report on the safety, 2007; Ryan et al., 2007). Kodym and Bujak revealed that aloin caused the development of stimulating contact dermatitis in skin-allergic patient whom received topical application of *Aloe arborescens Miller* (Kodym and Bujak, 2002).

Although a number of bioactive constituents of Aloe plant substantiate their use as the wound healing agents, it is believed that these constituents prompt healing in a concert action, rather than acting alone. The results of the current study show that two whole-leaf juice preparations, either from *Aloe ferox Miller* or *Aloe arborescens Miller*, have the positive effect on wound healing, antimicrobial action and dermal irritation response. The results provide the primary evidence of the medicinal property of Aloe whole-leaf juice for topical treatment of skin injury. However, further clinical trials regarding such claims are necessary before accurate conclusions regarding these heath benefits can be made.

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