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Preparation and Evaluation of *Annona glabra* L. Leaf Extract Contained Alginate Film for Burn Healing

Hoang Le Son[1*] and Nguyen Thanh Tram[2]

[1]School of Biotechnology, International University – Vietnam National University, HCMC, Vietnam.

Authors' contributions

This work was carried out in collaboration between all authors. Authors HLS and NTT designed the study. Author HLS managed the literature searches, wrote the protocol, and the first draft of the manuscript, read and approved the final manuscript. Author NTT prepared and evaluated the formulas, performed the animals test and statistical analysis.

Research Article

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ABSTRACT

Aims: To prepare and evaluate herbal wound dressing comprising of *Annona glabra* L. leaf extract and calcium alginate on experimental animal models.
Study design: Qualitative analysis for phytochemicals was carried out. Wound dressing material was formulated and characterized before the efficacy of formula was evaluated.
Place and Duration of Study: School of Biotechnology, International University, Vietnam National University, Ho Chi Minh City, between August, 2012 and May, 2013.
Methodology: Phytochemicals from ethanol leaf extract were screened by standard methods. Extract-loaded calcium alginate films were first dried cast from the gel formulations of 1.0%, 2.0%, 3.0%, 4.0%, and 0% (w/v) extract. The dried film morphologies and in vivo wound healing profiles were then investigated. Third-degree burn wounds were induced in Swiss albino mice divided into seven groups of 5 mice each. Groups I-V were given formula containing 1.0%, 2.0%, 3.0%, 4.0%, and 0% (w/v) extract, respectively. Group VI (negative control) received no treatment at all while group VII (positive control) was applied the standard dressing, Urgo Algoplaque (Laboratories Urgo).
Results: Phytoconstituents that were detected including flavonoids, glycosides, saponins,

**Corresponding author: Email: hlson@hcmiu.edu.vn;*

tannins, steroids, acidic compounds, and anthraquinones. There was a negligible difference in the physicochemical appearance of the prepared dressings. The topical application of these extract-loaded films with a dose of up to 4% extract accelerated significantly (P<0.001) wound healing process compared to the standard dressing Urgo Algoplaque. Groups I-IV were healed in a mean time of 16.8, 14.6, 11.6, and 11 days respectively, which were even faster than that of the standard dressing (18.4 days). The most prominent formula facilitated wound contraction without dermal irritation was the film impregnated 3.0% extract.

Conclusion: Administration of the *A. glara* contained dressing promotes burn healing as evidenced by decreased healing time and faster wound contraction. It could be stated that *A. glabra* leaves possess wound healing property.

Keywords: Annona glabra; calcium alginate; burn wound; wound dressing; wound healing.

1. INTRODUCTION

Burn wounds continue to be the most common accidental injuries. Not only burn injuries disfigure to the skin, but they can also cause severe consequences on body function. These injuries can be healed completely with minimal scarring [1]. The entire process of wound healing is a dynamic and intricate process which involve ordered cascade of events to restore the integrity of damaged tissue [2]. It involves different overlapping phases and processes including haemostasis, inflammation, proliferation, tissue remodeling, and formation of granulation tissue with angiogenesis. An array of cytokines and growth factors also coordinates the interaction between epidermal and dermal cells, the extra cellular matrix, controlled angiogenesis and plasma-derived proteins in order to facilitate a wound repair process [3,4]. Many local factors can impact the wound healing. Bacterial infection is the most important factor that can affect the development of wound. The most common bacteria with strong pathogenicity isolated from burn wound are *Pseudomonas aeruginosa* and *Staphylococcus aureus* [5]. T*hough wound healing is a biological process, selection of a suitable wound dressing is also* important in order to possibly and significantly reduce the healing time.

In general, an effectual wound dressing should maintain the most suitable environment at the wound dressing interface. Such optimum conditions include mechanical and bacterial protections, moisture balance, exudates management, gaseous and fluid exchange, biocompatibility, non-adhesion to the wound as well as removability without trauma. Based on these mentioned requirements, various wound care products are produced and can be categorized into three groups including passive, interactive, and active dressings. Passive wound dressings which are of the common covers on a wound, such as gauze, lint, nonstick and tulle. These dressings are permeable to bacteria that may adhere to the wound, cause trauma and pain on removing. Interactive wound dressings such as semi-permeable films/foam and amorphous hydrogels prevent bacterial permeation and allow gaseous exchange. Active or bioactive wound dressings include hydrofibers, hydrocolloids, collagens, and alginates. They maintain an appropriately moisturizing environment for wound bed and also absorb exudates. Among the bioactive dressings, calcium alginate dressings are viewed as being highly biocompatible and biodegradable [6-8].

Calcium alginate, an anionic linear polysaccharide extracted from brown seaweed, is viewed as being biocompatible, hydrophilic and biodegradable under normal physiological

conditions. It is composed of 1-4-α-L-guluronic acid (G) and β-D-mannuronic acid. Due to the presence of divalent calcium ions (Ca^{2+}), calcium alginate exhibits a positive charge and gelation characteristics [9,10]. Ca^{2+} acts as a cofactor for many blood-clotting enzymes and plays a key role in haemostasis after tissue damage [11]. The outer membrane of gram-negative bacteria contains lipopolysaccharides (LPS) that provide bacterial structural integrity and protect them from chemical attack. Ca^{2+} can breakdown lipopolysaccharides (LPS) of *Pseudomonas aeruginosa* [12]. Products from calcium alginate, which form gels upon contact with wound, have been used to control exudates, result in haemostasis and accelerate wound healing [9]. Despite its efficacy, the development of wound dressings has changed from passive to active types by impregnated with some herbal plants in recent years [8,13-15].

Healing power in plants has been discovered over the past many years. *Annonaceae* plants have long been used for treatment of diseases. *Annona glabra* Linn. (*A. glabra*) is in family *Annonaceae*, commonly known as pond apple, which is a tropical plant easily grown in fresh and brackish wetlands including Vietnam [16]. *A. glabra* was reported to contain numerous bioactive compounds which possess antibacterial, antifungal, insecticidal and cytotoxic properties [14,17]. The alcoholic *A.glabra* leaf extract was studied non-cytotoxic to normal human lymphocytes [16]. The bactericidal property of ethanolic extract prepared from pond apple leaves was reported against gram-negative bacteria that commonly found in infected burn wound such as *Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus* [17,5]. Although *A. glabra* leaves contain numerous bioactive compounds, their effects on burn wound have not been sufficiently known.

The purpose of this present contribution is to develop an alginate dressing with enhanced wound healing ability, through the use of phytochemicals contained in *A. glabra* leaves. This study was initiated to determine whether *A. glara* leaf extract-loaded alginate film may protect the wound from infection, keep moisturizing environment for wound bed, and thus promote the healing process. The physicochemical parameters of these films were analyzed and burn healing activity was evaluated using burn mouse model.

2. MATERIALS AND METHODS

2.1 Materials

Calcium alginate (CaAlg) and hydroxylpropyl cellulose (HPC) (1000000 M.Wt) were purchased from Sigma Chemical Co. (St. Gallen, CH, Germany). Glycerol, 99% ethanol (EtOH) and sodium carbonate (Na_2CO_3) were procured from Shanghai Demand Chemical Co., Ltd. Distilled water was used throughout.

2.2 Experimental Animals

Healthy Swiss mice *Mus musculus var. Albino* weighing 16-22 grams were procured from Pasteur Institute of Ho Chi Minh City. They were housed in clean cages and had free access to standard pallet diet and water *ad libitum*. During the experiment, mice were placed under a controlled environmental condition with 12 h of light and dark cycle.

2.3 Preparation of *Annona glabra* Leaf Extract

Matured fresh leaves of *A. glabra* were collected from Ho Chi Minh City, Vietnam. These leaves were first rinsed thoroughly with tap water, shaded dried for 5 days and pulverized into powdery form using an electric grinder. Plant extraction was done by cold maceration of 500 g in 80% ethanol with intermittent shaking at 2 h interval for 72 h. The filtrate obtained was concentrated using rotator evaporator. A highly non-polar solvent such as hexane was used to remove the chlorophyll out of the extract. The extract was then concentrated and preserved in glass petri dishes at 4°C prior to using for the phytochemical screening and preparing formula.

2.4 Phytochemical Screening

The ethanol leaf extract of *A. glabra* was subjected to phytochemical detection of major constituents such as alkaloids, flavonoids, glycosides, saponins, tannins, steroids, terpenoids, acidic compounds, anthraquinones, reducing sugars, and phlobatanins. This qualitative analysis was conducted as previously described by methods of Harbone [18], Trease and Evans [19].

2.5 Preparation of *A. glabra* Leaf Extract-Loaded Calcium Alginate Films

A mixed solution of 20 mL of Na_2CO_3 and CaAlg (4.0% w/v) [20] was combined with 1.2 mL of glycerol. The mixture was then added to HPC (1.0% w/v) [21] before blending with leaf extract (1.0% w/v, 2.0% w/v, 3.0% w/v, 4.0% w/v, and 0% w/v) to produce five different gel formulations F1 - F5 (Table 1). These were dried at 37°C for at least 24 h and *A. glabra* extract-loaded calcium alginate films were obtained after this stage.

Table 1. Formulations of the calcium alginate film containing *A. glabra* leaf extract

Formulation	Ingredients					
	CaAlg (g)	Leaf extract (g)	HPC (g)	Na_2CO_3 (mL)	Glycerol (mL)	Distilled water (mL)
F1	1.2	0.3	0.3	20	1.2	8.8
F2	1.2	0.6	0.3	20	1.2	8.8
F3	1.2	0.9	0.3	20	1.2	8.8
F4	1.2	1.2	0.3	20	1.2	8.8
F5	1.2	0	0.3	20	1.2	8.8

2.6 Characterization of *A. glabra* Leaf Extract-Loaded Alginate Films

2.6.1 Physicochemical tests of dressings

Prepared formulations were inspected for their morphology such as film thickness, uniformity of weight, peel adhesion properties, thumb tack test, folding endurance, percentage of elongation break test, percentage of moisture content, solvent loss, and water vapor permeability (WVP) were performed according to the relevant studies [8,22-23].

2.6.2 Skin irriration test

A slightly modified version of OECD Guidelines for Testing of Chemicals [24] was conducted to determine skin irritation. The formulation to be tested was applied on the shaved bare skin of healthy albino mice and covered with gauze patch. Test substance was removed after 24h, and dermal reactions were recorded and graded according to the method of Draize [25] as follows: 0.00: no irritation; 0.04 – 0.99: irritation barely perceptible; 1.00 – 1.99: flight irritation; 2.00 – 2.99 mild irritation; 3.00 – 5.99: moderate irritation; 6.00 – 8.00: severe irritation. The average of 24-72 hrs scores represents the final score.

2.7 In Vivo Wound Healing Studies

2.7.1 Animal grouping

Mice were randomly distributed into seven groups each containing 5 mice. Groups I-V were daily administered with formula F1, F2, F3, F4 and F5, respectively. Group VI served as negative control which received no treatment at all. Group VII served as positive control that was given the standard dressing *Urgo Algoplaque* (*Laboratories Urgo*).

2.7.2 Gross examination of the burn wound lesion

Mice were inflicted with burn wound as described by the previous procedure [26]. The dorsal surface of animals was shaved off one day prior to applying the burn making procedure. A rectangular bar with a cross sectional area of 2.0 cm^2 was heated over the open flame for 30s and pressed to the shaved surface of anesthetized animals for 20 s. After 2 days, the necrosis tissue was removed and formulas were applied once per day until complete healing. On the 4^{th}, 7^{th}, 10^{th}, 14^{th} and 21^{th} days, after having had treated, the wound appearance and wound contraction were monitored according to the relevant studies [8,13].

$$\% \text{ Wound contraction} = \frac{\text{Original wound area - Unhealed wound area}}{\text{Original wound area}} x100\% \quad [13]$$

2.7.3 Healing time

Time taken for full healing was measured by recording the days required for falling of eschar leaving no raw wound area behind [27]

2.7.4 Histological examination

Upon completion of the experiment, biopsies were taken from 6 mice (2 after burn injury, 2 in normal, and 2 in the group showed the fastest healing rate). Their regenerated tissue was then fixed in paraffin and stained using Hematoxylin and Eosin (H&E) method [13]. The epidermis and dermis layers, fibroblasts, keratinocytes, and collagen fibers were observed in order to evaluate the burn healing efficacy.

2.8 Statistical Analysis

Statistics is completely randomized designs for in vivo animal studies. Data were presented as the mean ± S.E.M analyzed by Student's t-test to examine the significant differences between experimental data. A $P < 0.001$ was considered significant. SPSS statistical

package (version 16) was used for analysis.

3. RESULTS AND DISCUSSION

3.1. Phytochemical Screening

The ethanol leaf extract of *A. glabra* indicated the presence of flavonoids, glycosides, saponins, tannins, steroids, acidic compounds, and anthraquinones while alkaloids, reducing sugars, terpenoids and phlobatanins were absent (Table 2).

Table 2. Phytochemistry of the ethanol leaf extract of *A. glabra*

Phytochemical constituents	Phytochemical test	Inference
Alkaloid	Dragendorff's test	-
	Mayer's test	-
Flavonoid	Ammonium test	+
	Aluminum chloride test	+
Glycoside	Fehling's test	+
Saponin	Emulsion test	+
	Frothing test	+
Tannin	Ferric chloride test	+
Steroid	Concentrated H_2SO_4 test	+
Terpenoid	Concentrated H_2SO_4 test	+
Acidic compound	Litmus paper	+
Anthraquinone	Borntrager's test	+
Reducing sugar	Fehling's test	-
Phlobatanin	HCl	-

Key: + (Presence); - (Absence)

3.2 Characterization of *A. glabra* Leaf Extract-Loaded Alginate Films

3.2.1 Physicochemical tests

The results of physicochemical properties including film thickness, uniformity of weight, peel adhesion, thumb tack, folding endurance, percentage of elongation break, percentage of moisture content, solvent loss, and WVP of all prepared dressings are shown in Table 3.

3.2.1.1 Film thickness and solvent loss

The thickness of the prepared dressings was around 0.47 mm with insignificant variation. All formulations complied with the WO 92/05755 [28] specifications for a dressing thickness of less than 0.5 mm. The films thickness uniformity was evidenced by low S.E.M value. The solvent loss values were ranged from 16.56±0.42 g for F5 to 18.30±0.27 g for F4. The solvent loss from the formulation could be of water and ethanol because only both solvents are likely to evaporate at drying temperature of 37°C.

Table 3. Physicochemical analysis of the formulas

Parameters	Formulation				
	F1	F2	F3	F4	F5
Thickness (mm)	0.45±0.02	0.49±0.01	0.47±0.01	0.43±0.02	0.43±0.02
Solvent loss (g)	18.07±0.54	17.16±0.12	17.79±0.64	18.30±0.27	16.56±0.42
Uniformity of weight (g)	4.49±0.01	4.79±0.02	5.12±0.01	5.27±0.01	4.21±0.02
Peel adhesion (N/25mm)	0.12±0.02	0.12±0.02	0.16±0.02	0.16±0.02	0.12±0.02
Thumb tack (N/cm^2)	0.22±0.04	0.20±0.04	0.22±0.03	0.24±0.02	0.22±0.02
Folding endurance (No')	91.60±0.81	92.00±0.95	93.4±0.51	92.80±0.66	90.40±0.51
Elongation break (%)	2.2±0.17	2.00±0.18	2.13±0.17	2.07±0.12	2.00±0.18
Moisture content (%)	9.48±3.53	11.16±1.34	9.26±2.80	14.66 ±4.97	23.28±3.47
WVP ($g/cm^2/h$)	0.03±0.009	0.04±0.006	0.03±0.010	0.05±0.017	0.06±0.009

Data are expressed as the mean ± S.E.M

3.2.1.2 Uniformity of weight

The films were ranged in weight from a mean of 4.21±0.02 g for F4 to a mean of 5.27±0.01 g for F2. There was a general trend of increasing weight with increasing of leaf extract concentration contained in formulations.

3.2.1.3 Peel adhesion properties

The measurement of peel adhesion (N/25 mm) was 0.12±0.02 for formulation F1, F2 and F5, while two other formulations showed peel adhesion value of 0.16±0.02. Two formulations showed higher peel adhesion value, F3 and F4, contained higher the amount of leaf extract. This could be considered as a relationship between the extract concentration and peel adhesion property.

3.2.1.4 Thumb tack test

The force required to remove thumb from adhesive varied from 0.20±0.04 N/cm^2 for F2 to 0.24±0.02 N/cm^2 for F4.

3.2.1.5 Folding endurance

The values of *folding endurance* were found to *vary* from 90.40±0.51 to 93.4±0.51 which shows the fairly strength and elasticity.

3.2.1.5 Elongation break

All formulations elongated about 2%. There was no relationship between elongation break and any other tested parameters.

3.2.1.7 Moisture content and WVP

Formulation F5 exhibited the highest percentage of moisture content and WVP (g) which were 23.28±3.47 and 0.06±0.009, respectively. F5 had approximately the double moisture content and WVP in comparison with the remains of film formulation.

3.2.2 Skin irritation test

The leaf extract-loaded alginate films induced dermal irritation is shown in Table 4. In irritated skin test, only F4 containing 4% leaf extract caused a slight erythema but no edema while other formulas caused non-irritation (Fig.1).

Table 4. Grading of skin reactions

Formu-lation	Reaction	24 Hours		72 hours		Irritation score	Evaluation
		Intact	Abraded	Intact	Abraded		
F1	Erythema	0	0	0	0	0	No irriation
	Edema	0	0	0	0		
F2	Erythema	0	0	0	0	0	No irriation
	Edema	0	0	0	0		
F3	Erythema	0	0	0	0	0	No irriation
	Edema	0	0	0	0		
F4	Erythema	0	1	0	2	5/24=0.2	Irritation barely perceptible
	Edema	0	1	0	1		

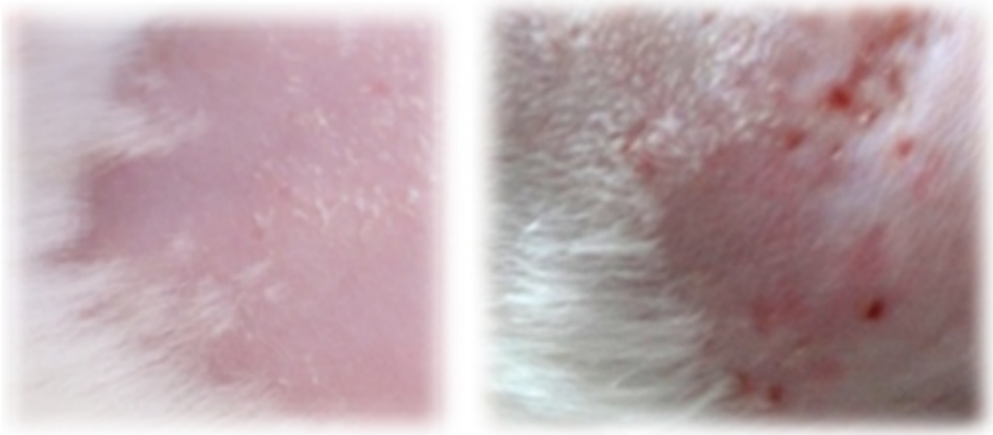

Fig. 1. Mouse skin before (a) and after (b) skin irritation test

3.3 Wound Evaluation

3.3.1 Wound appearance

Wound healing of mice was characterized by wound appearance on days 4th, 7th, 14th, and 21st, as indicated in Fig.2.

Days 1st - 4th [Fig. 2(1-4)]

Haemostasis and inflammation occur soon after the skin was damaged. Haemostasis is achieved under interaction between endothelial cells, platelets, and coagulation factors. Inflammatory response begins and causes erythema, swelling and fever which are often associated with pain around the wound site [3,4]. These phenomena were observed in group V, VI, and VII. Group VI and VII exposed minimal exudates. In contrast, no inflammation occured in groups I-V which received formula containing leaf extract. On day 4th, wound of group I-V contracted and formed scar tissue.

Day 7th [Fig. 2(5-8)]

Only wound bed of group VI covered with soft yellow slough occurred an inflammation. Wound of group VII was non-infected, however, an edema still presented. There were insignificant differences in wound appearance of group I, II, and V. These groups exhibited dried wound margin and purple tissue in wound base. In group III, and IV, a part of scar tissue was unattached.

Day 10th [Fig. 2(9-12)]

Wound exudates of group VI was seropurulent, resulting from bacterial infection [29]. Wound margins of group I, II, V, and VII turned to yellow firm tissue, but they were still attached. Eschars of group III, and IV completely fell. Red and healthy tissue was observed. However, wound of group IV was recognized an edema.

Day 14th [Fig. 2(13-16)]

After infected stage, yellow brown eschar was formed in wound of group VI. Firm eschar of group I, II, and V fell a half. Wound margin of group VII still attached. On days 11th - 14th period, wounds of group III and IV were completely healed with no scar.

Day 21th [Fig. 2(17-19)]

Burn area of group VI was in the process of being speedy recovered. Pink firm granulation tissues were steady formed. Wound healing process of group V and VII ended up with scar while group I and II were healed with no scar.

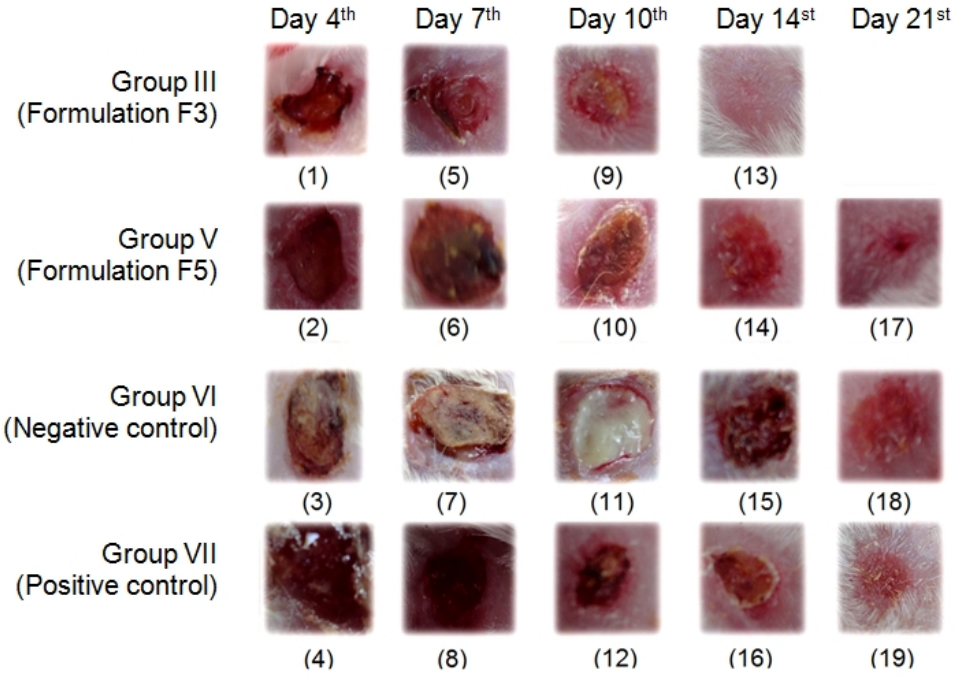

Fig. 2. Gross morphology of healing burn wound

3.3.2 Wound contraction and healing time

The percentage of wound contraction was found to increase in a time-dependent manner in all groups (Table 5). Significant higher rate (P<.001) of wound closure was observed in mice received *A. glabra* leaf extract contained alginate films (Groups I - IV) on the first week of wound as compared to that of having wound contraction with 0% leaf extract-contained alginate films (Group V), untreated (Group VI), and Urgo Algoplaque (Group VII). The faster rate of wound contraction reached 96% (Group III) and 97% (Group IV) by 10th day. In empty leaf extract-contained alginate films treated (Group V) animals, the wound contraction was markedly delayed (P<.001) throughout the study period in comparison with untreated mice (Group VI), and insignificant difference (P>.05) in correlation with Urgo Algoplaque treated animals (Group VII). In the present study, both formulations containing *A. glabra* leaf extract (F1-F4) and empty extract (F5) enhanced wound contraction; however, the injuries were healed faster in groups received formulations F1-F4. These results reflected that the composite film possessed good healing property than the tested remains.

Mice were given formulation F3 (Group III) and formulation F4 (Group IV) completed firstly the healing process within a mean of 11.6 ± 0.6 days and 11 ± 0.45 days (Table 6), respectively, and no significant difference was observed (P=.45). Group VII (Urgo Algoplaque treatment) was recovered slower than Groups I - IV (leaf extract contained alginate films administration) by 18.4 ± 0.24 days (Table 6).

Table 5. Effect of *A. glabra* leaf extract contained alginate films on burn mouse model

Groups	Percent wound contraction (%)				
	4th day	7th day	10th day	14th day	21th day
I	42.22±0.82[abc]	72.48±0.59[abc]	85.76±0.29[abc]	96.83±0.62[abc]	_
II	46.44±0.45[abc]	75.65±0.36[abc]	88.52±0.37[abc]	98.34±1.08[abc]	_
III	55.42±0.52[abc]	77.42±0.32[abc]	96.16±0.99[abc]	_	_
IV	55.58±0.17[abc]	77.06±0.38[abc]	96.99±1.27[abc]	_	_
V	35.79±1.13[a]	64.44±0.46[a]	83.29±0.96[a]	91.76±0.37[a]	_
VI	20.89±0.98	45.90±2.11	64.03±0.64	85.61±0.42	99.05±0.49
VII	36.95±0.63	63.01±0.55	81.33±0.63	90.13±0.40	_

Key: (-) Completed closure.
Data are expressed as the mean ± S.E.M, (n=5); P<.001 was considered significantly different in comparison with [a]control; [b]Urgo Algoplaque; [c]Formulation F5

Table 6. Time for burn healing

Group	Healing time (days)
I	16.8 ± 0.49
II	14.6 ± 0.40
III	11.6 ± 0.6
IV	11.0 ± 0.45
V	18.8 ± 0.37
VI	22.8 ± 0.73
VII	18.4 ± 0.24

Data are expressed as the mean ± S.E.M, (n=5)

It was found that with an increase in the *A. glabra* leaf concentration, the healing time was considerably reduced. The medicinal value of the prepared formulations was probably due to the combination of calcium alginate and *A. glabra* leaf extract. Calcium alginate is an effective haemostat, generally well tolerated by body tissues. The calcium-sodium ion exchange which takes place when the dressing is in contact with wound exudate allowing calcium ions to be released into the wound and assisting in haemostasis [11]. The wound healing property of the extract lies in bioactive phytochemical constituents that can promote wound healing process [26]. These plant phenolics were traditionally contributed to heal wounds. Tannins, saponins and flavonoids are known to be effective in astringent for wound and prevention of infections. Saponins possess antioxidant property while tannins act as free radical scavengers, enhancing wound contraction and elevated rate of epithelialization [30-32].

3.3.4 Histological examination

The photomicrographs of wound from group III were taken at specific intervals for visual comparison. The normal skin tissues composed of three main layers including epidermis (E), dermis (D), and hypodermis (H) [Fig. 3(1)]. The cellular components including keratinocytes, fibroblast, adipose cells, and angioblast were found in this sample [Fig. 3(4)]. When thermal injury occurred, it damaged the entire epidermis, dermis, and part of hypodermal layer. Therefore, the dye could bind to the debris giving a violet color [Fig. 3(2)]. The presence of lymphocytes [Fig. 3(6)] showed evidence for inflammation phase which occurred in the early stage of wound repair. Wound on 11th day was being healed with the cover of E, D, and H layers [Fig. 3(3)] and the cellular components were in the process of being recovered similar

to the normal state [Fig. 3(5)]. However, some hair follicle was destroyed and could not be recovered. Based on the clinical features and the histological results, third-degree burn wounds were created successfully. Burn wounds were typically dry, leathery, black brown, and painless. The dermis and subcutaneous fat were damaged, resulting in local inflammatory response. During wound healing process, the formation of cellular components such as lymphocytes, fibroblast, keratinocytes, and collagen marked a good restoration of tissue. Lymphocytes remain in the wound for a week and then decrease in number if none noxious stimulant of further inflammation persists. Keratinocytes form a basement membrane filter against environmental damage such as pathogens, and fibroblasts, the main component in the extracellular matrix, results in collagen formation [26].

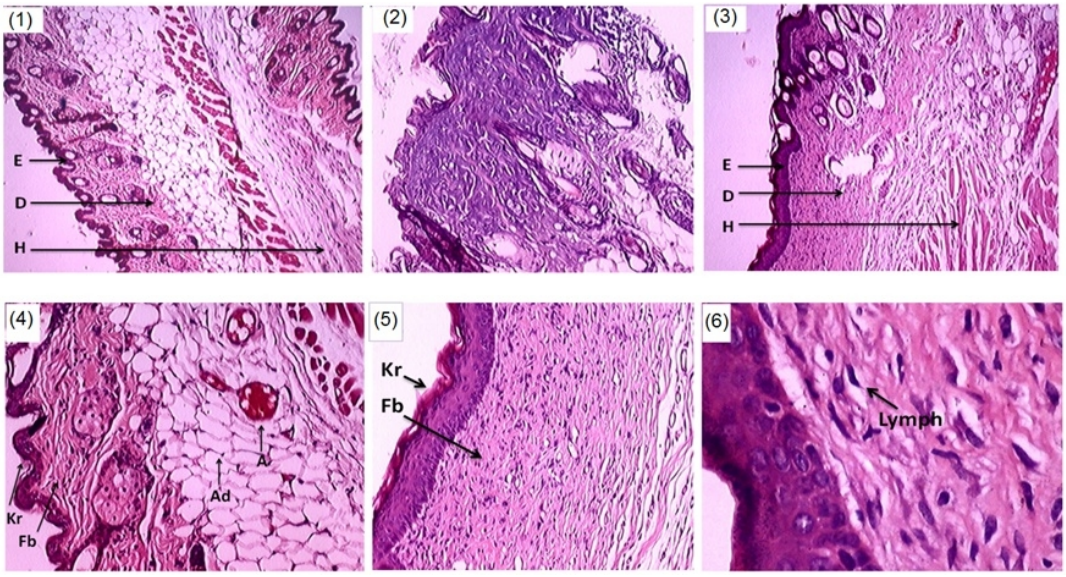

Fig. 3. Histological features of mouse skin tissues before and after burn injury.
(1) Normal skin tissues composed of epidermis (E), dermis (D), and hypodermis (H); (2) Burned skin tissues; (3) Healing skin tissues of group III at day 11; (4) The cellular components of normal skin tissues included keratinocytes (Kr), fibroblast (Fb), adipose cells (Ad), and angioblast (A); (5) the cellular components and (6) the presence of lymphocytes in healing skin tissue

4. CONCLUSION

The results demonstrate that the alginate films impregnating *A. glabra* leaf extract exerted wound healing activity, and even enhanced the rate of wound contraction. It is believed that the phytochemicals in the leaf extract play a key role in the promotion of healing process. At a dose of 3.0% (w/v) *A. glabra* leaf extract, burn wound was recovered well without dermal irritation. Formulae containing extract denoted the good outcome of burn healing; however, clinical trials are needed to validate the potential of these wound healing dressings.

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CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments were conducted in accordance with animal use ethics as accepted internationally.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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