Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2022 Apr 12; 10(A):634-643.
https://doi.org/10.3889/oamjms.2022.9140
eISSN: 1857-9655
Category: *A - Basic Sciences*
Section: *Pharmacolgy*

The Effect of *Eurycoma longifolia* Jack Tongkat Ali Hydrogel on Wound Contraction and Re-Epithelialization in *In Vivo* Excisional Wound Model

Maryam Riyadh Yaseen Al-Bayati[1]*, Ghasak G. Faisal[2], Azliana Abd Fuaat[1], Khairunisa Ahmad Affandi[1], Muhammad Adil Zainal Abidin[3]

[1]Department of Pathology and Laboratory Medicine, Faculty of Medicine, International Islamic University, Selangor, Malaysia; [2]Department of Fundamental Dental and Medical Sciences, Faculty of Dentistry, International Islamic University, Selangor, Malaysia; [3]Department of Community Medicine, Faculty of Medicine, International Islamic University, Selangor, Malaysia

Edited by: Mirko Spiroski
Citation: Al-Bayati MRY, Faisal GG, Fuaat AA, Affandi KA, Abidin MAZ. The Effect of *Eurycoma longifolia* Jack Tongkat Ali Hydrogel on Wound Contraction and Re-Epithelialization in *In Vivo* Excisional Wound Model. Open Access Maced J Med Sci. 2022 Apr 12; 10(A):634-643. https://doi.org/10.3889/oamjms.2022.9140
Keywords: Wound healing; *Eurycoma longifolia* Jack (Tongkat Ali); Gas chromatography-mass spectrometer analysis
***Correspondence:** Maryam Riyadh Yaseen Al-Bayati, Department of Pathology and Laboratory Medicine, Faculty of Medicine, International Islamic University, Selangor, Malaysia. E-mail: alhadetheemaryam@gmail.com
Received: 27-Feb-2022
Revised: 21-Mar-2022
Accepted: 02-Apr-2022

Funding: This research did not receive any financial support
Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Wound management is one of the significant health problems throughout the world. Medicinal plants have been used widely in wound management. *Eurycoma longifolia* Jack which is known as Tongkat Ali (TA) is a tropical medicinal plant in South East Asian countries.

AIM: The aim of the study was to investigate the effect of (TA) hydrogel on wound contraction and re-epithelialization in excisional wound model in rats.

METHODS: Twenty male Sprague Dawley rats were divided into four groups each group contained five rats (n = 5). Animal treatment groups are formed as: Untreated (−ve) control, Hydrocyn® aqua gel (+ve), vehicle hydrogel, and (TA) hydrogel. A full-thickness circular excisional wound was created on the dorsal back of each rat. The wounded area was measured and photographed on days 3, 6, 9, 12, 15, and 18 post wounding to determine the percentage of wound contraction and re-epithelialization.

RESULTS: (TA) hydrogel showed significant increase in the percentage of wound contraction by 43.38% compared with the other groups ($p = 0.032$, $p < 0.050$) during the first interval (inflammatory phase). Although in the later healing stages (proliferative and remodeling) and re-epithelialization, our test group (TA) hydrogel did not show statistically difference with the other groups yet it was comparable to medically certified wound healing agent.

CONCLUSION: (TA) hydrogel significantly accelerated the wound healing process during the early stage, the inflammatory stage. Whereas during the later healing stages and re-epithelialization, it showed almost the same effect of Hydrocyn® aqua gel.

Introduction

Skin is the biggest organ in the body with diversified functions, such as defense against microbial infestation, chemical, radiological and mechanical impacts, regulating the temperature of the body, and playing a crucial role in the functioning of the nervous system. Therefore, according to its size and important functions, the skin must be treated and heal rapidly [1].

Wound can be defined as the destruction of the anatomical and functional integrity of the skin [2]. In general, the wound healing is a dynamic and complex process, it consists of four overlapping stages which are; hemostasis, inflammation, proliferation, and tissue remodeling. The inflammatory stage is one of the crucial steps of the healing cascade. Acute inflammation plays a substantial role in wound healing based on homeostasis restoration [3]. The main purpose of the inflammatory phase is to clear out debris, remove dead and necrotic tissue and phagocytize pathogens from the wound bed to prepare the wound environment for effective healing [4]. Neutrophils and monocyte-macrophages cooperate as partners in space and time during induction, progression, and resolution of the inflammatory phase. This myelomonocytic interplay is an essential element of wound healing [5]. After that, the proliferative phase initiates by replacing the wound with healthy and strong granulation tissue via angiogenesis, fibroplasia, and re-epithelialization. The final stage of the wound healing cascade is maturation, also known as remodeling. The essential goal of this stage is the reorganization, devastation, and regeneration of the extracellular matrix (ECM) to gain maximum tensile strength [6]. However, persistent or extended inflammation can increase the activity of proteolytic enzymes in wounds thus demolishing growth factors and the ECM in wounds, which is very necessary for migration and proliferation of fibroblasts and endothelial cells [7]. Therefore, extended inflammation can prolong or impair the healing process.

With the rapid development in economic and industrial sectors, cutaneious injuries can occur easily every day [3]. Non-healing or chronic wounds cause severe effects on the life quality of patients as well as causing an economic load on health-care institutions [8]. Although the availability of various therapies for managing patients with acute and chronic wounds for the past decades, these therapies are usually expensive and accompanied by undesirable side effects. Therefore, there is a great tendency to search for alternative wound healing agents particularly from medicinal plants [3], [9].

Recently, plants and their by-products have attracted remarkable attention as a source of therapeutic agents to treat wounds. Medicinal plants or phytochemicals have played a significant role in curing diseases throughout mankind's history. Today, most people are still relying on traditional remedies to meet their primary health care needs. Optimal topical wound agents must be safe, non-toxic, and biocompatible [10].

Eurycoma longifolia Jack (Tongkat Ali [TA]) is one of the most important medicinal plants in Malaysia; it belongs to the Simaroubaceae family. Many studies have shown that (TA) has various medicinal properties. All the parts of the plant are very effective especially the roots. Conventionally, the root extract of (TA) has been used as an anti-inflammatory and analgesic medicine [11]. Scientifically, many studies have proved that (TA) root extract has antioxidant, cytotoxic, antimalarial, antipyretic, anti-inflammatory, antitumor, and aphrodisiac properties, as well as, it has been used in the curing of various conditions such as fatigue, impotence, fever, and high blood pressure [11], [12], [13]. Tran *et al*. that proved the inhibitory effect of *E longifolia* Jack (TA) root on NF-κB inflammatory pathway [14]. In addition, the previous studies showed that *E. longifolia* Jack (TA) extract has antimicrobial effects against candida albicans, streptococcus mucus, *Staphylococcus aureus*, *Bacillus cereus*, and *S.* typhi [15], [16], [17]. All these pharmacological activities and the effectiveness of *E. longifolia* Jack (TA) are attributed to the presence of important phytochemicals especially in the root [18].

According to the reports of phytochemical screening of *E. longifolia* Jack (TA) root extracts have shown the presence of quassinoids, phenolic compounds, flavonoids, terpenoids, alkaloids, cardiac glycosides, proteins, and mucopolysaccharides [19], [20]. Many studies have displayed that any medicinal plant with those phytochemicals having great potential for enhancing and accelerating wound healing process. The most common mechanisms behind the phytochemical-mediated enhanced wound healing cascade are their polytropic sources as anti-microbial, anti-inflammatory, and antioxidant effects [21], [22]. Many recent studies have proved that the effectiveness of medicinal plants in acceleration wound healing process due to availability of important phytochemicals, essential oils and volatile compounds which have antioxidant and anti-inflammatory activities [10], [23].

So far there is no study to investigate the effectiveness of *E. longifolia* Jack (TA) as a wound healing agent. In our previous study, the root of *E. longifolia* Jack (TA) was authenticated by microscopic examination, extracted by Soxhlet technique using absolute ethanol and prepared it in a hydrogel as a suitable dosage form for the *in vivo* wound application [18]. Our previous study was the first one that documented the possibility of preparation of *E. longifolia* Jack (TA) roots in a hydrogel for wound application.

Hydrogels have been widely used as wound healing agents due to its ability in protection the wound environment and absorption the exudates from the wound due to their consistent capacities for water retention and superior biocompatible features [24]. Furthermore, hydrogels serve as a depot that can carry the medicated molecules for controlled release [25].

The aim of the current study was to investigate the topical effect of *E. longifolia* Jack (TA) hydrogel on the percentage of wound contraction and re-epithelialization in excisional wound model in Sprague Dawley rats.

Materials and Methods

Chemicals and reagents

Dichloromethane (DCM) was used in gas chromatography-mass spectrometer (GC-MS) analysis, ethanol 95%, denatured was from HmbG (Hamburg, Germany), normal saline Sodium Chloride (NaCl) 0.9% Irrigation Solution BP from Elder Elite (Penang, Malaysia), Ketamine hydrochloride (100 mg/mL), and Xylazine Hydrochloride (100 mg/mL) from the ilium (Australia), Hydrocyn® aqua gel 15 g from Bactiguard AB (Tullinge, Sweden).

Qualitative and quantitative analysis of volatile compounds and essential oils of ethanol root extract of Eurycoma longifolia Jack (TA) by GC-MS

Recently, the studies of (GC-MS) analysis have been widely applied for the examination of herbal medicines, as this method has been considered a precious method for the analysis of plants' phytochemicals [26]. Ethanol extract of *E. longifolia* Jack (TA) roots was subjected to GC-MS analysis for the determination of volatile compounds and essential oils. The GC-MS analysis was done using a Clarus TM 680/GC SQ8T/MS, GC/MS Perkin Elmer, Elite-5MS (30 m × 0.25 mm × 0.25 um) Capillary Column (Brand: Perkin Elmer). The sample was diluted in DCM Dichloromethane with the ratio of 1:100 and filtered using a Syringe Filter. The

apparatus was set to a starting temperature of 30℃, the temperature was raised up to 280°C, at the rate of an increase of 10°C/min and held for 10 min. The total running time was 35 min. Mass spectral scan range was set at 30–500 m/z. The chemical compounds were identified tentatively by matching their mass spectra data with those from the mass spectral search program for the NIST/EPA/NIH Mass Spectral Library (NIST MS Search version 2.0). The expected element, retention time area, and percentage of the area were established.

Experimental animals

The animal study was done to investigate the effect of *E. longifolia* Jack (TA) hydrogel in the healing of excisional wound model in Sprague–Dawley rats. The animal study was conducted on 20 Male Sprague–Dawley rats (160–180 g). The animals were ordered from an animal supplier, A SAPPHIRE ENTERPRISE in Selangor Darul Ehsan, Malaysia. The rats were kept individually to avoid any injuries by interactions in clean polypropylene cages in the animal retention house of the Department of Basic Medical Science, Faculty of Pharmacology, International Islamic University Malaysia (IIUM) in a well-ventilated room with 12-h light/dark cycles at 25℃ of temperature and 55%–60% relative humidity. All rats were allowed free access to pellets and clean water *ad libitum*. Animals were given 1 week for acclimatization before starting the experiment.

Ethical approval

The animal study was approved by the Institutional Animal Care and Use Committee (IACUC) of IIUM, approval number: IIUM/504/14/2/IACUC. Animal care and experimental protocols were conducted according to the guidelines endorsed by the Guide for the Care and Use of Laboratory Animals (NIH publication No: 85–23, revised in 1985).

Acute dermal irritation study

The test was conducted according to the guidelines of the Organization for Economic Co-operation and Development (OECD) for testing chemicals to get information on health hazard that possible to arise from skin's exposure to test substance [27]. The Objective of this test in our study was to assess the irritation profile of *E. longifolia* Jack (TA) hydrogel and vehicle hydrogel (xanthan) when applied topically on intact shaved skin of Sprague–Dawley rats to ensure its safety before placing it on the excisional wound. There was not acute dermal irritation test for Hydrocyn® aqua gel since it is a recognized and medically certified wound agent and certified gel for topical application.

After 1 week of acclimatization, 10 male Sprague–Dawley rats 180–200 g were used in this test. They were divided into two groups, five rats each for *E. longifolia* Jack TA hydrogel and vehicle hydrogel (2% xanthan) groups. An area of 4 cm^2 of the dorsal back of each rat was prepared 1 day before the test by shaving the hair and sterilizing the hairless skin with ethanol alcohol. On the test day, the same amount of each hydrogel was applied on the hairless skin with non-irritating dressing plasters to ensure skin contact. The animals were checked for the developing of erythema and edema in accordance with the scoring system of Draize dermal irritation (0 = no erythema or no edema; 1 = slight erythema or edema; 2 = precisely marked erythema or little edema; 3 = moderate to severe erythema or moderate edema, and 4 = severe erythema or edema) at grading intervals of 24, 48, and 72 h of hydrogel application [28]. As well as the primary irritation index (PII) was calculated, then was classified in accordance with the Draize method of classification using the PII scoring as non-irritant (if PII <0.5), slightly irritant (if PII <2), moderately irritant (if PII _ 2–5), and severely irritant (if PII >5) [29].

Surgical procedure of excision wound model and study design

The excisional wound model as designated by Morton and Malone was conducted in this study [2], [30]. After weighing each rat by METTLER TOLEDO balance, the animals were anesthetized by intraperitoneal injection with 10 ml of anesthesia cocktail. The anesthesia was prepared by mixing 8.75 mL of ketamine 100 gm/ml concentration with 1.25 mL of xylazine 100 gm/mL concentration. The dose was calculated according to body weight. 0.1 mL of the cocktail was injected into the peritoneal for every 100 gm of body weight. The dorsal hair of the rats was removed by a sterilized electrical shaver. Then, the shaved area was cleaned with 70% v/v ethanol. The size and location of the wound were determined by marking pen, engineering stencils (plastic model), and caliper as shown in Figure 1. A full-thickness circular wound of 15 mm in diameter and 2 mm depth was created at the dorsal interscapular region of each rat by sterile surgical scissors, scalpel size (3), blade size (15), and toothed forceps. The wound was located at the intrascapular region on the rat's back in order to keep it away from licking, so the wound was protected from rats' oral pathogen [2].

Wound creation started with the incision of the marked skin by surgical scalpel blade then excised the skin by surgical scissors and toothed forceps as shown in Figure 2. Hemostasis was obtained by compressing the wound with sterile gauze. After the bleeding was stopped, the wound was measured again with calliper in longitudinal and transverse dimensions to ensure the accuracy of wound size as shown in Figure 3. The 20 wounded

rats were divided into four groups, each group contained five rats (n = 5) according to the formula in Sample Size Calculation in Animal Studies Using Resource Equation Approach [31]. Experimental animals were arranged as shown in Figure 4 in to four groups: Group 1 untreated (−ve) control, Group 2 Hydrocyn® aqua gel (+ve) control, Group 3 vehicle hydrogel (2% w/w xanthan), and Group 4 *E. longifolia* Jack (TA) hydrogel (xanthan-based hydrogel containing 0.12%w/w TA). All treatments were applied twice daily morning and evening with the amount of 1 gm of each treatment from the 1st day of wound creation for the whole experiment time. Wounds were left undressed in the wound area and the percentage of wound contraction was evaluated on days 0, 3, 6, 9, 12, 15, and 18 of the experiment.

Quantification of wound area and calculation of the percentage of wound contraction

On days 3, 6, 9, 12, 15, and 18, the area of the wound for each rat in each group was measured in two dimensions, longitudinal, and transverse using caliper. The wound areas were calculated using the ellipse area formula [32]:

$$A = a \times b \times \pi$$

Where:

A is the area of an ellipse

a represents the major radius of the ellipse

b represents the minor radius of the ellipse

π is a constant having value of 3.1415.

The healing in wound area was monitored and evaluated, indicating the rate of wound contraction and epithelialization period. The evaluated surface area was used to calculate the percentage of wound contraction, taking the initial size of the wound as 100% as shown below [3], [33]:

$$\%\ \text{Wound contraction} = \frac{\left(\text{wound area on } 1^{st}\text{day} - \text{wound area on the day } [n]\right)}{\text{Wound area on } 1^{st}\text{day}} \times 100$$

Epithelialization period measurement

Subsidence of scab without fresh wound behind was considered as the endpoint of entire epithelialization and the days needed for this were counted as a period of epithelialization [33].

Figure 1: Surgical and measuring instruments were used in creation excisional wound model on Sprague–Dawley rats. (a) Instruments used for the excisional wound creation: calliper, plastic model (engineering stencil), marker, scalpel no (3), blade no (15), surgical scissor, tissue forceps. (b) Determination 2 mm depth on surgical blade, the depth of the excisional wound. (c) Determination the size and location of the wound at intrascapular dorsal region

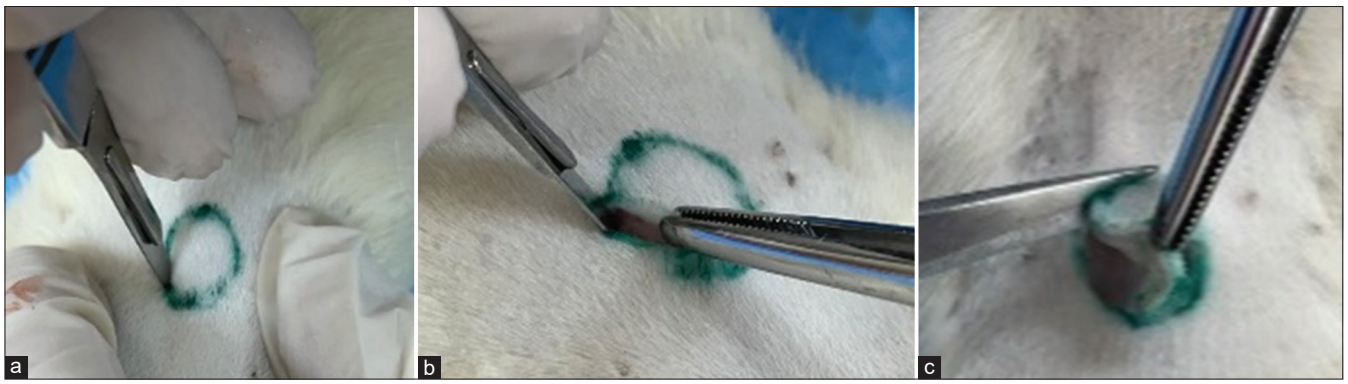

Figure 2: Steps of surgical procedure to create excisional wound model (15 mm diameter and 2 mm depth) on the dorsal back of Sprague–Dawley rat at intrascapular region. (a) Incise the marked skin with scalpel and blade. (b) Holding the incised skin with surgical toothed forceps. (c) Eexcise or resect the skin with tissue forceps and surgical scissor

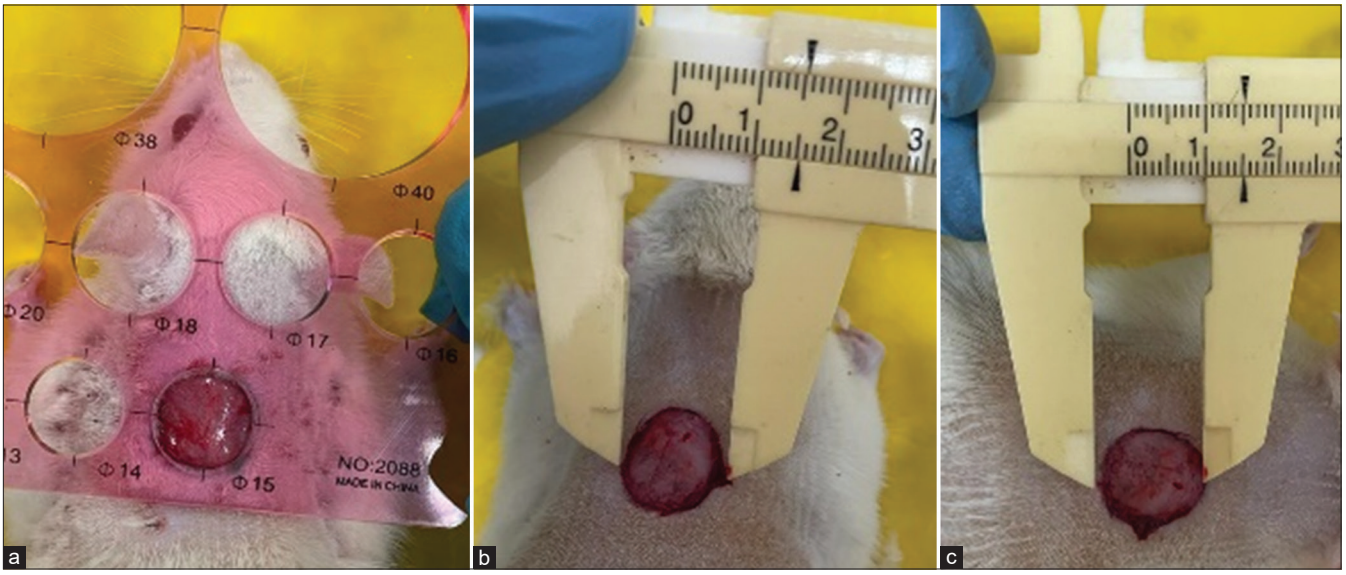

Figure 3: Checking the size of the wound in two dimensions after creation on wounding day (Day 0). (a) Checking the wound size after excision. (b) Size of the wound in Transverse dimension 15 mm on wounding day. (c) Size of the wound in longitudinal dimension 15 mm on wounding day

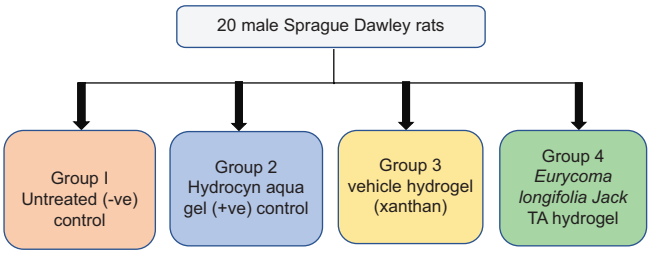

Figure 4: Experimental groups of Sprague–Dawley rats for wound healing study

Statistical analysis

The data were entered and analyzed using (SPSS) version 20. Kruskal–Wallis test was used to compare the median of wound contraction and duration of epithelialization between the four groups and the data were expressed as median (interquartile range [IQR]) and p was considered statistically significant with a value less than 0.05 ($p < 0.05$).

Results

Qualitative and quantitative analysis of volatile compounds and essential oils of Eurycoma longifolia Jack (TA) ethanol extract by GC-MS analysis

According to the qualitative and quantitative analysis of ethanol extract of *E. longifolia* Jack (TA) by GC-MS chemical analysis, the crude extract of the root contains many of the volatile compounds and essential oils with antioxidant and anti-inflammatory effects which are necessary for the wound healing process as shown in Table 1.

Table 1: Interpreted data of gas chromatography-mass spectrometer analysis of the ethanol extract of *Eurycoma longifolia* Jack (Tongkat Ali) roots

Number	RT	Area	Percentage area	Expected element
1	9.04	2170158	12.98	Pyranone
2	10.40	4395376	26.30	5-Hydroxymethylfurfural
3	12.39	307094	1.84	1-Hexadecene
4	13.18	3416378	20.44	Guanosine
5	13.86	998540	5.97	2,4-Di-tert-butylphenol
6	14.84	778531	4.66	Hexadecenoic acid, ethyl ester
7	17.06	452828	2.71	1-Hexadecanol
8	18.77	2045677	12.24	Hexadecanoic acid
9	19.07	3218245	19.26	Palmitic acid, ethyl ester
10	20.46	2853788	17.07	Oleic acid
11	20.70	1116765	6.68	Oleic acid, ethyl ester
12	22.67	1714027	10.26	Hexanedioic acid, bis (2-ethylhexyl) ester
13	23.90	609040	3.64	Diisooctyl phthalate

Remark (if any): The chemical compounds were identified tentatively by matching their mass spectra data with those from the mass spectral search program for the NIST/EPA/NIH Mass Spectral Library (NIST MS Search version 2.0). RT: Retention time.

Skin irritation testing

The primary skin irritation index of both *E. longifolia* Jack (TA) and vehicle (xanthan) hydrogels was calculated and the results were 0.00. The hairless skin of all tested rats showed a score of 0 of acute dermal irritation on the hydrogel application as shown in Figure 5 and Tables 2, 3. The tested areas were observed for 3 days and no erythema, edema, or irritation were recorded during the entire period of study. Hence, both tested hydrogels were non-irritant to the rat's skin.

Excision wound model

Quantification of wound area and measurement of wound contraction

The macroscopic evaluation was conducted to monitor the healing process through photographs. Figure 6 shows illustrative pictures of the retraction of the wound area during the experiment over days (0, 3, 6, 9, 12, 15, and 18). Table 4 illustrates the size of

the wound area over 6 times follow-up post wounding. Table 5 shows the percentages of wound contraction between the four experimental groups over the same 6 times follow-up. Based on the Kruskal–Wallis test, our test hydrogel, *E. longifolia* Jack (TA) hydrogel showed significant increase in the percentage of wound contraction on day 3 compared with the other three groups ($p = 0.032$, $p < 0.05$, n=5). Whereas on following intervals (6, 9, 12, and 15) *E. longifolia* Jack (TA) hydrogel displayed higher percentage of wound contraction than the other groups, however the difference was not sizable enough to show statistical significance.

Re-epithelialization

Table 6 shows the duration of re-epithelialization (falling of the scab) for all four groups. The period of re-epithelialization was indicated by a number of days.

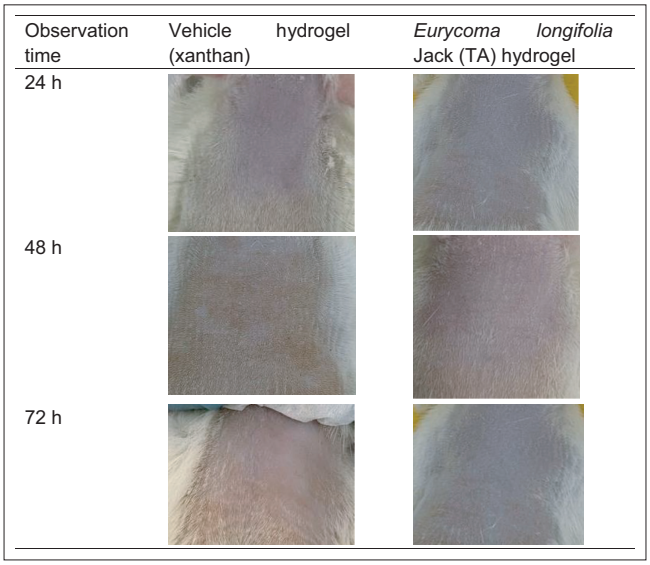

Figure 5: Photographic observation of acute dermal irritation study of the rats for vehicle (xanthan) and (Tongkat Ali) hydrogels over 3 days

Table 2: Acute dermal irritation calculation for vehicle hydrogel (2% w/w xanthan)

Skin reaction	Observation time (h)	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Total
Erythema formation	24	0	0	0	0	0	0
	48	0	0	0	0	0	0
	72	0	0	0	0	0	0
Edema formation	24	0	0	0	0	0	0
	48	0	0	0	0	0	0
	72	0	0	0	0	0	0

Total of mean score: 0, PII: 0, Classification: Non-irritant. PII: Primary irritation index.

Table 3: Acute dermal irritation calculation for *Eurycoma longifolia* Jack (Tongkat Ali) hydrogel (xanthan-based hydrogel containing 0.12% w/w Tongkat Ali)

Skin reaction	Observation time (h)	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Total
Erythema formation	24	0	0	0	0	0	0
	48	0	0	0	0	0	0
	72	0	0	0	0	0	0
Edema formation	24	0	0	0	0	0	0
	48	0	0	0	0	0	0
	72	0	0	0	0	0	0

Dermal responses were scored according to OECD guidelines. Mean score of dermal responses=Total score of erythema formation+total score of edema formation/3, PII=Mean score at 24 h+mean score at 48 h+mean score at 72 h/3. Total of mean score: 0, PII: 0, Classification: Non-irritant. PII: Primary irritation index, OECD: Organization for Economic Cooperation and Development.

The shortest time for falling of the scab was for the *E. longifolia* Jack (TA) hydrogel group with 13 ± 1.00, whereas 14.6 ± 0.54, 13.8 ± 1.78 and 14.2 ± 1.30 days were for the untreated (−ve) control, Hydrocyn® aqua gel (+ve) control and vehicle hydrogel, respectively. However, according to the Kruskal–Wallis test, there was no significant difference in the re-epithelialization period between the four groups p - value= 0.204 ($p < 0.05$, $n = 5$).

Discussion

Although the natural skin has an innate ability to drive auto regeneration after the injury, its capability could be obstructed under conditions such as extensive acute wounds and chronic wounds. Therefore, once the healing cascade got delayed, it would lead to microbial infections and other complications. Thus, discovering new therapeutic approaches to accelerate wound healing process is demanded [3].

Nowadays, medicinal plants with natural antioxidant and anti-inflammatory properties have dragged much attention in wound treatment because they can stimulate wound healing through modifying the immune response at the molecular level [34].

E. longifolia Jack (TA) is one of the most popular medicinal plants in South East Asian countries. The roots of *E. longifolia* Jack (TA) are the most important part of the plant. Many studies have shown that the root has anti-malarial, aphrodisiac, anti-osteoporosis, antioxidant, and anti-inflammatory effects [35]. All these pharmacological effects are attributed to the availability of bioactive compounds, which are concentrated in the root.

In our previous study, we prepared the ethanol extract of *E. longifolia* Jack (TA) roots in a hydrogel as a wound healing agent for *in vivo* study [18]. In the current study, we investigated the effect of *E. longifolia* Jack (TA) hydrogel on the percentage of wound contraction and duration of re-epithelialization of excisional wound model in male Sprague–Dawley rats.

To ensure the safety application of *E. longifolia* Jack (TA) hydrogel and the vehicle on excisional wound, we performed the acute dermal irritation test in accordance with OECD guidelines [27]. The results of both (TA) and vehicle hydrogels were non-irritant as shown in Figure 4 and Tables 2, 3. *E. longifolia* Jack (TA) queues extract was tested by Ahmad *et al*., which was safe like our alcohol extracted *E. longifolia* Jack (TA) [35]. This suggests extraction method has no effect on the safety profile of *E. longifolia* Jack (TA). Besides, vehicle hydrogel (xanthan) was very safe to apply on rat's skin and there was no irritation. The results of testing the vehicle hydrogel were in agreement with

Table 4: The results of wound area calculation of 4 studying groups over 6 follow-up times

Wound area	Days	Untreated (− ve) control	Hydrocyn® aqua gel (+ ve) control	Vehicle hydrogel (xanthan)	*Eurycoma longifolia* Jack (TA) hydrogel
Wound area after wounding day (mm^2)	Day 0	176.71	176.71	176.71	176.71
	Day 3	119.84 ± 12.89	117.96 ± 8.66	113.25 ± 7.97	100.05 ± 8.53
	Day 6	63.92 ± 9.74	68.33 ± 10.28	64.03 ± 6.06	62.82 ± 8.67
	Day 9	21.99 ± 3.14	35.65 ± 22.84	16.48 ± 1.75	22.61 ± 2.62
	Day 12	5.18 ± 1.72	23.30 ± 16.17	8.24 ± 3.86	6.28
	Day 15	0.00 (healed)	8.24 ± 2.4	4.71 ± 0.98	0.00 (healed)
	Day 18	0.00 (healed)	0.00 (healed)	0.00 (healed)	0.00 (healed)

TA: Tongkat Ali.

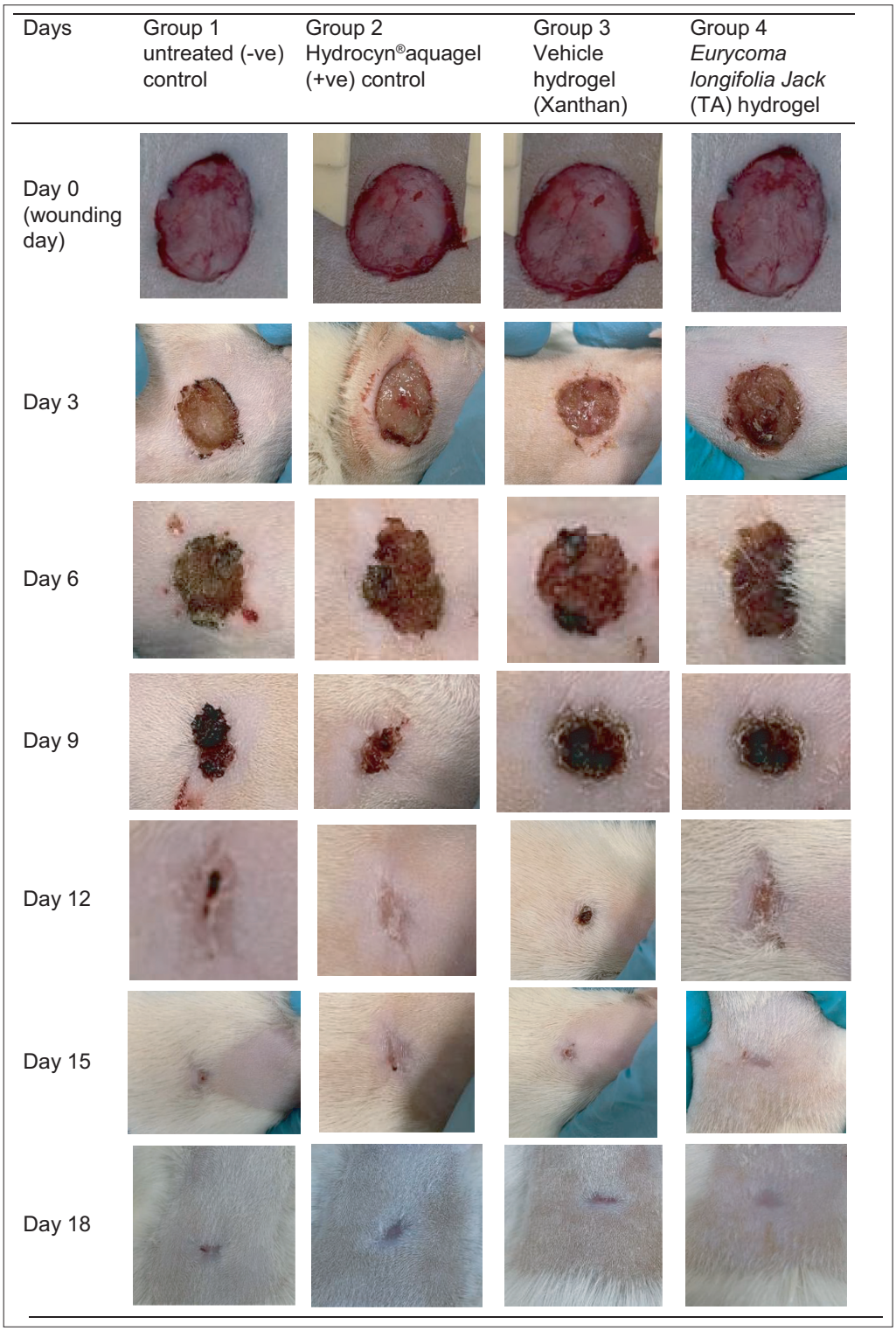

Figure 6: Gross images of excisional wounds of four experimental groups over 18 days post-treatment: Group 1: untreated (−ve) control, Group 2: Hydrocyn® aqua gel (+ve) control, Group 3: vehicle hydrogel (xanthan), and Group 4: Eurycoma longifolia Jack (Tongkat Ali) hydrogel

Table 5: The effect of *Eurycoma longifolia* Jack (Tongkat Ali) hydrogel on the percentage of wound contraction among the four study groups over days: 3, 6, 9, 12, and 15

Wound percentage	Days	Untreated (− ve) control (%)	Hydrocyn® aqua gel (+ ve) control (%)	Vehicle hydrogel (xanthan) (%)	*Eurycoma longifolia* Jack (TA) hydrogel (%)	*p*
The percentage of wound contraction (%)	Day 3	32.18	33.24	35.91	43.38	0.032
	Day 6	63.82	61.33	63.76	64.45	0.123
	Day 9	87.55	79.82	90.67	87.20	0.093
	Day 12	97.06	86.81	95.33	96.44	0.584
	Day 15	100	95.33	97.33	100	0.222

Values are median (IQR), n = 5, p < 0.05 Kruskal–Wallis test. TA: Tongkat Ali, IQR: Interquartile range.

Table 6: The period of re-epithelialization among the four different studying groups

Treatment groups	Period of re-epithelialization (days) mean ± STD
(− ve) control, no treatment	14.6 ± 0.54
(+ ve) control, Hydrocyn® aqua gel	13.8 ± 1.78
Vehicle hydrogel, Xanthan	14.2 ± 1.30
Eurycoma longifolia Jack (TA) hydrogel	13 ± 1.00

STD: Standard deviation, TA: Tongkat Ali.

many previous studies which have been conducted on the importance of natural polysaccharide incorporation in topical applications. Singhvi *et al.* documented that one of the xanthan applications in pharmaceutical industries is its incorporation in the preparation of hydrogel for wound healing applications [36].

Wound contraction is defined as the centripetal locomotion of the edges in a full-thickness wound model. Therefore, wound contraction is an indication for re-epithelialization, ECM production, formation of new vasculature (angiogenesis) and cellular proliferation and differentiation to facilitate wound closure [8]. In this context, *E. longifolia* jack (TA) hydrogel group showed the highest percentage of wound contraction during the early stage of wound healing (day 3) with 43.38% which was statistically significant compared with Hydrocyn® aqua gel (+ve) control group, vehicle hydrogel (xanthan) group, and untreated (−ve) control group with p value = 0.032 ($p < 0.05$, $n = 5$). Whereas on the following intervals (days 6, 9, 12, and 15), our testing hydrogel did not show statistical significance in the wound contraction compared with the other groups. It is speculated that the significant effect of *E. longifolia* Jack (TA) hydrogel during the early stage (inflammatory) of wound healing could be attributed to the availability of phytochemicals with anti-inflammatory and antioxidant properties.

According to the interpreted data of GC-MS analysis of the ethanol extract of *E. longifolia* Jack (TA) roots (Table 1) many anti-inflammatory/antioxidant agents were extracted. 5-hydroxymethylfurfural was the highest concentration in the extract with the 26.30% area followed by palmitic acid with 19.26% and 17.07% for oleic acid (OA). Recently, 5-HMF have been found to show significant effects on human health as an antioxidant, an anti-inflammatory and anti-sickling agent [37]. Kong *et al.* showed the anti-inflammatory/antioxidant effects of 5-HMF in accelerating wound healing process in excisional wound model in rats through stimulating wound contraction, increasing fibroblast proliferation, enhancing collagen production, and increasing growth factors expression such as vascular endothelial growth factor [3]. Another study documented that 5-HMF was applied an anti-inflammatory effect in lipopolysaccharide-catalyzed inflammatory response through suppressing the MAPK, NF-κB and Akt/mTOR pathways [38]. Besides 5-HMF, OA is another anti-inflammatory/antioxidant agent in TA extract. Rodrigues *et al*. reported that the topical application of OA or taken orally can regulate the immune response and controls the inflammatory phase in wound healing cascade [39]. Gallelli *et al*. showed in his pilot study that was conducted on diabetic patients, the topical application of (OA) is safe and effective in healing diabetic foot ulcer [40]. It is important to regulate the level of inflammation in the wound because prolonged inflammation can lead to cell dysfunction and chronic diseases. The result of day 3 post wounding agrees with the results of other medicinal plants with antioxidant/anti-inflammatory effects based - wound agents [3], [34].

Whereas the reason for lacking the significant effect of *E. longifolia* Jack (TA) hydrogel during the later stage (proliferative and remodeling) of healing could be attributed to its antiproliferative effect. Abdulelah *et al.* had proved that the root of *E. longifolia* Jack (TA) has antiproliferative properties with anticancer and cytotoxic effect against lymphocytes of human chronic myelocytic leukemia [41]. However, the metabolic pathway of cancer cell is relatively different from that of normal cell. Normal cell proliferation, migration, and programmed death (apoptosis) are stimulated by growth-factor signaling pathways, those pathways often become impaired in cancer [42].

Epithelialization is an essential element of cutaneous wound healing used as an indication for successful healing of the wound. Re-epithelialization depends mainly on keratinocyte proliferation; without re-epithelialization a wound cannot be considered successfully healed. In all types of chronic wounds there is impaired re-epithelialization [43]. In this context, we investigated the effect of *E. longifolia* Jack (TA) hydrogel on duration of re-epithelialization (number of days for the scab falling). The shortest duration of re-epithelialization was shown by *E. longifolia* Jack (TA) hydrogel group (13 ± 1.00); however, the difference was not sizable enough to show statistical significance. Again, it is speculated that the lack of statistical significance could be attributed to the antiproliferative effects of *E. longifolia* Jack (TA) root, since re-epithelialization depends on keratinocytes proliferation.

It is worth noting that in the later healing stages and re-epithelialization there was no statistical

significance difference between our testing agent and Hydrocyn® aqua gel, which is a certified and medically recognized hydrogel for wound healing. Therefore, *E. longifolia* Jack (TA) hydrogel might serve as a potential therapeutic agent in injuries with hyperinflammation.

The current study is the first one that investigated the possible effects of *E. longifolia* Jack (TA) hydrogel in stimulating wound healing process. Our results confirmed the significant effects of topical application of (TA) hydrogel during the early stage and its comparable effects with the medically certified wound healing agent during the later stage of wound healing process. Our study has some limitations. It would be ideal to conduct on bigger sample size; our sample size (five rats per group) was a relatively small.

Conclusion

This study showed that *E. longifolia* Jack (TA) hydrogel is significantly accelerated the wound healing process during the early stage (inflammatory phase) of wound healing. Whereas during the later healing stages, it showed almost the same effect of medically certified wound healing agent. It is thought that the availability of volatile compounds and essential oils with antioxidant and anti-inflammatory effects play a positive role in the healing cascade. Further studies are required to investigate the effect of *E. longifolia* Jack (TA) hydrogel on wounds with prolonged inflammatory phase such as chronic and burn wounds.

References

1. Salehi M, Zamiri S, Samadian H, Ai J, Foroutani L, Ai A, *et al*. Chitosan hydrogel loaded with Aloe vera gel and tetrasodium ethylenediaminetetraacetic acid (EDTA) as the wound healing material: *In vitro* and *in vivo* study. J Appl Polym Sci. 2021;138(16):50225. https://doi.org/10.1002/app.50225
2. Bektas N, Şenel B, Yenilmez E, Özatik O, Arslan R. Evaluation of wound healing effect of chitosan-based gel formulation containing vitexin. Saudi Pharm J. 2020;28(1):87-94. https://doi.org/10.1016/j.jsps.2019.11.008
3. Kong F, Fan C, Yang Y, Lee BH, Wei K. 5-hydroxymethylfurfural-embedded poly (vinyl alcohol)/sodium alginate hybrid hydrogels accelerate wound healing. Int J Biol Macromol. 138:933-49. https://doi.org/10.1016/j.ijbiomac.2019.07.152
   PMid:31351961
4. Han G, Ceilley R. Chronic wound healing: A review of current management and treatments. Adv Ther. 2017;34(3):599-610. https://doi.org/10.1007/s12325-017-0478-y
   PMid:28108895
5. Cañedo-Dorantes L, Cañedo-Ayala M. Skin acute wound healing: A comprehensive review. Int J Inflamm. 2019;2019:3706315. https://doi.org/10.1155/2019/3706315
   PMid:31275545
6. Ajith G, Goyal AS, Rodrigues FC, Thakur G. Natural polysaccharides for wound healing. In: Food, Medical, and Environmental Applications of Polysaccharides. Elsevier: The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom; 2021. https://doi.org/10.1016/b978-0-12-819239-9.00019-1
7. Yaghoobi R, Kazerouni A, Kazerouni O. Evidence for clinical use of honey in wound healing as an anti-bacterial, anti-inflammatory antioxidant and anti-viral agent: A review. Jundishapur J Nat Pharm Prod. 2013;8(3):100-4. https://doi.org/10.17795/jjnpp-9487
   PMid:24624197
8. Boakye YD, Agyare C, Ayande GP, Titiloye N, Asiamah EA, Danquah KO. Assessment of wound-healing properties of medicinal plants: The case of Phyllanthus muellerianus. Front Pharmacol. 2018;9:1-12. https://doi.org/10.3389/fphar.2018.00945
9. Nguyen VL, Truong CT, Nguyen BC, Van Vo TN, Dao TT, Nguyen VD, *et al*. Anti-inflammatory and wound healing activities of calophyllolide isolated from *Calophyllum inophyllum* Linn. PLoS One. 2017;12(10):e0185674. https://doi.org/10.1371/journal.pone.0185674
   PMid:29020015
10. Sharma A, Khanna S, Kaur G, Singh I. Medicinal plants and their components for wound healing applications. Future J Pharm Sci. 2021;7(1):53. https://doi.org/10.1186/s43094-021-00202-w
11. Rehman SU, Choe K, Yoo HH. Review on a traditional herbal medicine, eurycoma longifolia Jack (Tongkat Ali): Its traditional uses, chemistry, evidence-based pharmacology and toxicology. Molecules. 2016;21(3):331. https://doi.org/10.3390/molecules21030331
    PMid:26978330
12. Ruan J, Li Z, Zhang Y, Chen Y, Liu M, Han L, *et al*. Bioactive constituents from the roots of *Eurycoma longifolia*. Molecules. 2019;24(17):1-16. https://doi.org/10.3390/molecules24173157
13. Varghese CP, C, A., Jin, S. C., Lim, Y. J., & Keisaban, T. Antioxidant and anti-inflammatory activity of *Eurycoma longifolia* Jack, a traditional medicinal plant in Malaysia. Int J Pharm Sci Nanotechnol. 2013;5(4):1875-8. https://doi.org/10.37285/ijpsn.2012.5.4.7
14. Tran TV, Malainer C, Schwaiger S, Atanasov AG, Heiss EH, Dirsch VM, *et al*. NF-κB inhibitors from *Eurycoma longifolia*. J Nat Prod. 2014;77(3):483-8. https://doi.org/10.1021/np400701k
    PMid:24467387
15. Alloha IB, Aziz NA, Faisal GG, Abllah Z, Arzmi MH. Effects of *Eurycoma longifolia* jack (Tongkat Ali) alcoholic root extract against oral pathogens. Pharmacogn J. 2019;11(6):1299-302. https://doi.org/10.5530/pj.2019.11.201
16. Faisal GG, Zakaria SM, Najmuldeen GF. *In vitro* antibacterial activity of *Eurycoma longifolia* Jack (Tongkat Ali) root extract. Int Med J Malaysia. 2015;14(1):77-81. https://doi.org/10.31436/imjm.v14i1.460
17. Faisal GG, Zakaria SM, Najmuldeen GF, Al-Ani IM. Antifungal activity of *Eurycoma longifolia* jack (Tongkat ali) root extract. J Int Dent Med Res. 2016;9(1):70-4.
18. Yaseen MR, Faisal GG, Fuaat AA, Affandi KA, Alallam B, Nasir MH. Preparation of *Euyrycoma longifolia* Jack (E.L) Tongkat Ali (Ta) root extract hydrogel for wound application. Pharmacogn J. 2021;13(6):1456-1463. https://doi.org/10.5530/PJ.2021.13.185
19. Abubakar BM, Salleh FM, Wagiran A. Chemical composition of *Eurycoma longifolia* (Tongkat Ali) and the quality control of its herbal medicinal products. J Appl Sci. 2017;17(7):324-38. https://doi.org/10.3923/jas.2017.324.338
20. Khanam Z, Wen CS, Bhat IU. Phytochemical screening

and antimicrobial activity of root and stem extracts of wild *Eurycoma longifolia* Jack (Tongkat Ali). J King Saud Univ Sci. 2015;27(1):23-30. https://doi.org/10.1016/j.jksus.2014.04.006

21. Shah A, Amini-Nik S. The role of phytochemicals in the inflammatory phase of wound healing. Int J Mol Sci. 2017;18(5):1068. https://doi.org/10.3390/ijms18051068
22. Barku VY. Wound Healing: Current Perspectives: London: IntechOpen Limited; 2019. p. 56-8.
23. Umar NM, Parumasivam T, Toh SM. An overview of cutaneous wounds and the beneficial roles of medicinal plants in promoting wound healing. Pharm Sci. 2021;27(4):489-502. https://doi.org/10.34172/ps.2021.16
24. Dumville JC, Stubbs N, Keogh SJ, Walker RM. Hydrogel dressings for treating pressure ulcers. Cochrane Database Syst Rev. 2015;2:CD011226. https://doi.org/10.1002/14651858.CD011226

    PMid:25914909
25. Almeida JF, Ferreira P, Lopes A, Gil MH. Photocrosslinkable biodegradable responsive hydrogels as drug delivery systems. Int J Biol Macromol. 2011;49(5):948-54. https://doi.org/10.1016/j.ijbiomac.2011.08.010

    PMid:21871915
26. Mary AP, Giri S. GC-MS Analysis of bioactive compounds *Achyranthes aspera*. World J Pharm Res 2018;7(1):1045-56. https://doi.org/10.20959/wjpr20181-10540
27. OECD 404. Guidelines for the Testing of Chemicals. Acute Dermal Irritation/Corrosion. OECD Guidelines for the Testing of Chemicals, Section 4 Health Effects. OECD; 2015. p. 1-8.
28. Bourke AR, Draize HJ. The Appraisal of Chemicals in Foods, Drugs and Cosmetics, Association of Food and Drug Officials of the United States. Austin, Texas: CiNii Articles; 1959. p. 49-52.
29. Banerjee S, Chattopadhyay P, Ghosh A, Pathak MP, Singh S, Veer V. Acute dermal irritation, sensitization, and acute toxicity studies of a transdermal patch for prophylaxis against (±) anatoxin-a poisoning. Int J Toxicol. 1972;32(4):308-13. https://doi.org/10.1177/1091581813489996

    PMid:23696561
30. Morton JJ, Malone MH. Evaluation of vulneray activity by an open wound procedure in rats. Archi Int Pharm Thera. 1972;196(1):117-26.

    PMid:5059357
31. Arifin WN, Zahiruddin WM. Sample size calculation in animal studies using resource equation approach. Malays J Med Sci. 2017;24(5):101-5. https://doi.org/10.21315/mjms2017.24.5.11

    PMid:29386977
32. Jørgensen LB, Sørensen JA, Jemec GB, Yderstræde KB. Methods to assess area and volume of wounds a systematic review. Int Wound J. 2016;13(4):540-53. https://doi.org/10.1111/iwj.12472

    PMid:26250714
33. Demilew W, Adinew GM, Asrade S. Evaluation of the wound healing activity of the crude extract of leaves of *Acanthus polystachyus* Delile (Acanthaceae). Evid Based Complement Altern Med. 2018;2018:2047896. https://doi.org/10.1155/2018/2047896
34. Comotto M, Saghazadeh S, Bagherifard S, *et al*. Breathable hydrogel dressings containing natural antioxidants for management of skin disorders. J Biomater Appl. 2019;33(9):1265-76. https://doi.org/10.1177/0885328218816526

    PMid:30961462
35. Ahmad N, Teh BP, Halim SZ, Zolkifli NA, Ramli N, Muhammad H, *et al*. *Eurycoma longifolia* infused coffee an oral toxicity study. Nutrients. 2020;12(10):3125. https://doi.org/10.3390/nu12103125

    PMid:33066137
36. Singhvi G, Hans N, Shiva N, Dubey SK. Xanthan gum in drug delivery applications. In: Natural Polysaccharides in Drug Delivery and Biomedical Applications. Amsterdam, Netherlands: Elsevier Inc.; 2019. https://doi.org/10.1016/B978-0-12-817055-7.00005-4
37. Shapla UM, Solayman M, Alam N, Khalil MI, Gan SH. 5-Hydroxymethylfurfural (HMF) levels in honey and other food products: Effects on bees and human health. Chem Central J. 2018;12(1):35. https://doi.org/10.1186/s13065-018-0408-3
38. Kong F, Lee BH, Wei K. 5-Hydroxymethylfurfural mitigates lipopolysaccharide-stimulated inflammation via suppression of MAPK, NF-κB and mTOR activation in RAW 264.7 cells. Molecules. 2019;24(2):275. https://doi.org/10.3390/molecules24020275

    PMid:30642099
39. Rodrigues HG, Vinolo MA, Magdalon J, Vitzel K, Nachbar RT, Pessoa AF, *et al*. Oral administration of oleic or linoleic acid accelerates the inflammatory phase of wound healing. J Investig Dermatol. 2012;132(1):208-215. https://doi.org/10.1038/jid.2011.265
40. Gallelli G, Cione E, Serra R, Leo A, Citraro R, Matricardi P, *et al*. Nano hydrogel embedded with quercetin and oleic acid as a new formulation in the treatment of diabetic foot ulcer: A pilot study. Int Wound J. 2020;17(2):485-90. https://doi.org/10.1111/iwj.13299

    PMid:31876118
41. Abdulelah FM, Abdulbaqi MR, Jaafar ZM. *Ex-vivo* anticancer evaluation of tongkat ali roots extract against lymphocyte cell line of human CML patient. Syst Rev Pharm. 2021;12(1):1206-11.
42. Intlekofer AM, Finley LW. Metabolic signatures of cancer cells and stem cells. Nat Metab. 2019;1(2):177-88. https://doi.org/10.1038/s42255-019-0032-0
43. Pastar I, Stojadinovic O, Yin NC, Ramirez H, Nusbaum AG, Sawaya A, *et al*. Epithelialization in wound healing: A comprehensive review. Adv Wound Care. 2014;3(7):445-64. https://doi.org/10.1089/wound.2013.0473

    PMid:25032064