ORIGINAL ARTICLE

Improved Quality of Healed Wounds with Topical Application of *Eurycoma Longifolia* Jack Root Extract Hydrogel

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ABSTRACT

INTRODUCTION: Collagen is the key component of the extracellular matrix that plays a critical role in the strength and quality of the healed wound. *Eurycoma longifolia* Jack root extract (TA) has been documented as an anti-inflammatory, antioxidant, and antimicrobial agent that may improve the quality of healed wounds. The objective of this study is to investigate the effect of topical application of TA hydrogel on the quality of the healed skin in rats. **MATERIALS AND METHODS:** Twenty male Sprague Dawley rats were grouped into 4 groups: Negative control, Hydrocyn® aqua gel positive control, vehicle (Xanthan) hydrogel, and *Eurycoma longifolia* Jack (TA) hydrogel. Treatments were applied twice daily starting on wounding day until day 21. An excisional wound was created on the back of 20 rats. Tissue samples of the healed skin were collected for histological examination by measuring the epidermal and dermal thicknesses and evaluating the collagen fibre quality by VVG stain. **RESULTS:** The TA hydrogel group had the thickest newly formed epidermis compared with the other experimental groups. For the dermal thickness, compared with the vehicle (xanthan) hydrogel group, TA hydrogel, and Hydrocyn aqua® gel positive control groups showed significantly increased thickness with *p* values 0.020 and 0.045, respectively. Histologically TA hydrogel group showed a significant increase in mixed-oriented collagen fibres, and fascicular collagen bundles and showed profound collagen density. **CONCLUSION:** TA hydrogel improved the quality of healed skin by increasing the epidermal/dermal thicknesses and enhancing the quality of newly produced collagen fibres. It can be considered a promising and effective wound-healing agent.

Keywords
Wound Healing, Eurycoma longifolia jack, collagen, dermal/epidermal thickness

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INTRODUCTION

Wounds refer to damage to the coherence of the skin's layers as a result of physical, chemical, or thermal injuries. Wounded tissues are characterized by lacking the functional and anatomical solidarity of the living organ.[1] Wound healing is a complicated biological process characterized by multiple cellular and biological events to restore the missing layers of the injured tissue.[2] Impairment in the regulation in any stage of the wound healing process retards healing and may lead to several skin pathologies, such as non-healing or chronic wounds.[3] The quality of healed wounds is a fundamental aspect of wound care. Wounds that delay in healing will heal weakly and will be prone to complications such as wound dehiscence and herniation.[4] Consequently, methods are sought that could improve the quality of healed wounds through the application of different types of dressing. Time reduction and avoiding inappropriate consequences are the main mechanisms for improving the wound healing process. Antibiotics, antiseptics, and sloughing agents have been utilised to hasten the healing process and decrease the inappropriate consequences. However, these agents are confronted with limitations due to their undesirable side effects and high costs.[5] Currently, research is trending to harness natural resources such as medicinal plants in managing and treating many kinds of wounds because of their availability, nontoxicity,

biocompatibility, and safety.[6,7] About 70% to 95% of the population in most developing nations and 70% to 90% of people in well-developed countries utilise orthodox medicine in their initial healthcare to manage their medical problems.[8] The key to the treatment of skin injury is to provide the outermost barrier, the epidermis, prevent infection, stop bleeding, moisturise and relieve pain.[9] *Eurycoma longifolia* Jack is native to Southeast Asian countries such as Thailand, India, Malaysia, and Vietnam. The roots of this plant are used by local folks in Malaysia as an aphrodisiac, to improve libido and energy, for hypertension and fever treatment. Many studies have confirmed that the root and root bark of *Eurycoma longifolia* Jack (TA) have many pharmacological effects such as anticancer, antimalarial, anti-inflammatory, and antioxidant properties.[10,11]

As well as the ethanol extract of TA roots, has been confirmed in previous studies to show antibacterial and antifungal effects.[12,13,14] All these pharmacological effects are assigned to the presence of important phytochemicals such as quassinoids, alkaloids, terpenoids, tannins, polysaccharides, glycosides, phenolic compounds, and other important bioactive compounds which are heavily concentrated in the roots.[10,11] In our previous study, we prepared the ethanol extract of *Eurycoma longifolia* Jack (TA) roots in a hydrogel for *in vivo* wound healing studies.[15] The current study aims to explore the effect of the topical application of TA hydrogel on the quality of the healed skin by measuring the epidermal and dermal thicknesses and evaluating the orientation of collagen fibres in the excisional wound model in Sprague Dawley rats.

MATERIALS AND METHODS

Animal and Housing

20 adult male Sprague Dawley rats of 160-180 g weight were used in this study and were handled carefully. The animal study was approved by The Institutional Animal Care and Use Committee of International Islamic University Malaysia (IACUC–IIUM) [approval number: IIUM/504/14/2/IACUC]. All details regarding animal care and housing are illustrated in our previous publication.[16]

Study Design and Surgical Procedure

The excisional wound model as designated by Morton and Malone was conducted in this study (Morton and Malone 1972; Bektas et al. 2020). After administering the anaesthesia to rats, a full-thickness circular wound of 15 mm×15 mm in diameter and 2mm depth was created surgically at the dorsal interscapular region of each rat. All details regarding the surgical procedure and calculation of the number of rats in each group are illustrated in our previous publication (Al-Bayati et al. 2022). All the 20 wounded rats were arranged into 4 studying groups [n=5]: Group 1 untreated (negative control), Group 2 Hydrocyn® aqua gel (positive control) control, Group 3 vehicle hydrogel (2% w/w xanthan) and Group 4 *Eurycoma longifolia* Jack (TA) hydrogel (xanthan-based hydrogel containing 0.12%w/w TA) as shown in. After getting haemostasis on a wounding day, all the types of treatments (Hydrocyn aqua gel, Vehicle hydrogel, and TA hydrogel) were applied twice daily for 21 days.

Histopathologic Examination

On day 21 after wound creation, rats were anesthetized and biopsies of healed skin were collected and processed by staining with H&E and Verhoeff's Van Gieson stain (VVG) to investigate histologically the effect of (TA) hydrogel on the collagen formed in the healed skin by histopathologic evaluation of collagen fibres characteristics and measurement of epidermal and dermal thicknesses. The thickness of the newly formed epidermis and dermis was measured using Leica Aperio ImageScope - Pathology Slide Viewing Software 12.4.3 on histologic sections stained by H&E stain.

The characteristics of collagen fibers in healed skin were evaluated semi-quantitatively by staining the tissue with VVG stain and observing 5 HPF in each slide. The collagen fibers were observed for the following characteristics; orientation, pattern, and density, Figure 7. Collagen density was assessed into 'minimal' where collagen fibers were sparse and widely spaced, 'moderate' where collagen fibers were loosely packed and 'profound' where collagen fibers were tightly packed. The data was analysed using a Chi-Square statistical analysis test to compare the percentage of fields having each score in the

different studied groups.

RESULTS

Histopathologic evaluation of the healed skin on day 21 post-wounding included investigating the effect of the topical application of TA hydrogel on the quality of the healed skin in terms of the epidermal and dermal thicknesses and collagen fibers characteristics in the healed skin.

A. Measurement of Epidermal and Dermal Thicknesses of Healed Skin

Figure 1 shows the measurement of the epidermal/ dermal thickness in the studied groups. The average thicknesses of the newly formed epidermis in untreated (negative control), Hydrocyn aqua® gel (positive control), vehicle (xanthan), and *Eurycoma longifolia* Jack (TA) hydrogels were 67.92 µm, 90.78 µm, 113.41 µm, and 118.05 µm respectively as shown in table1 that *Eurycoma longifolia Jack* (TA) hydrogel group showed the thickest newly formed epidermis compared with the other experimental groups, however, the difference was not sizable enough to show significance, p=0.055 Kruskal - Wallis test.

Whereas the average thicknesses of the newly generated dermis in untreated (negative control), Hydrocyn aqua® gel (positive control), vehicle (xanthan) and *Eurycoma longifolia* Jack (TA) hydrogels were 876.84 µm, 1182.7 µm, 543.3 µm and 1106 µm respectively as shown in table 1 that *Eurycoma longifolia* Jack (TA) hydrogels and Hydrocyn aqua® gel (positive control) control groups showed significantly increase in dermal thicknesses compared with the vehicle (xanthan) hydrogel group with p=0.020 and 0.045 respectively, Kruskal-Wallis test (n=5) *p<0.05. However, there is no statistically significant between *Eurycoma longifolia* Jack (TA) hydrogels and negative control and positive controls, our testing substance showed the smallest standard error in all the results, which means its effect on all the rats was similar and consistent.

The values are mean ± standard deviation (SD) (n=5). Kruskal - Wallis test. No statistical significance between the four groups p>0.05; P = 0.055.for epidermal thickness. *Eurycoma longifolia* Jack (TA) hydrogel group and Hydrocyne aqua gel positive control significantly increase

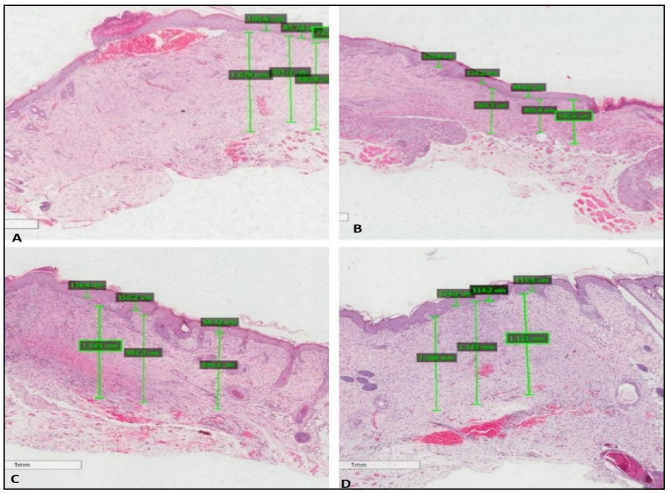

Figure 1: Measurement of epidermal and dermal thicknesses of healed skin on day 21. *Leica* Aperio ImageScope - Pathology Slide Viewing Software 12.4.3, scale 1mm was used in measuring three fields at 20x magnification. Slides were scanned by Aperio CS2 image capture device. A: Negative control, B: Vehicle (xanthan), C: Hydrocyn® aqua gel (positive control) control D: *Eurycoma longifolia* Jack (TA) hydrogel.

the newly formed dermal thickness compared with vehicle (xanthan) hydrogel group with p=0.020 and 0.045 respectively. *Kruskal-Wallis* test (n=5) *p<0.05

Table 1: Thickness of the newly formed epidermis and dermis (µm) on Day 21 post-wounding in the 4 experimental groups

Groups	Epidermal thickness	Dermal thickness
Untreated negative control	67.928 ± 34.50 µm	876.84 ± 151.7 µm
Hydrocyn aqua® gel positive control	90.78 ± 41.44 µm	1182.7 ± 435.4 µm
Vehicle (xanthan) hydrogel	113.41 ± 2.13 µm	543.3 ± 27 µm
Eurycoma longifolia Jack (TA) hydrogel group	118.05 ± 9.86 µm	1106 ± 23.8 µm

B. Histopathological Evaluation of Collagen Fibers Characteristics by VVG Stain

Figure 2 shows the histological picture of the different studied groups stained with VVG stain to show the characteristics of collagen fibers.

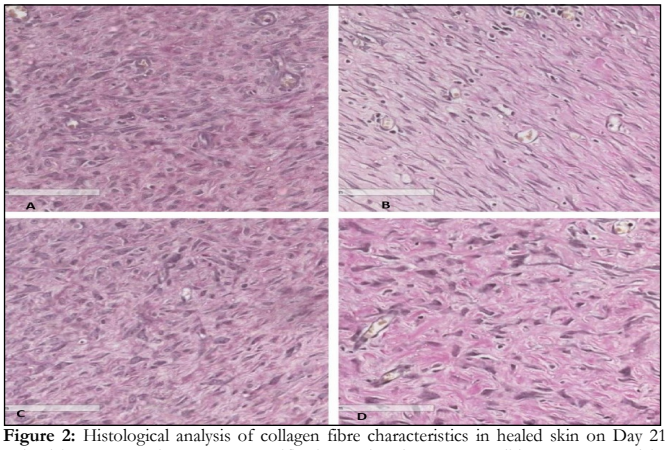

Figure 2: Histological analysis of collagen fibre characteristics in healed skin on Day 21 stained by VVG stain. At 40x magnification and scales 200 µm. Slides were scanned by Aperio CS2 image capture device. A: Negative control, B: Hydrocyn® aqua gel positive control, C: Vehicle (xanthan), D: Eurycoma longifolia Jack (TA) hydrogel.

Table 2 shows the results for collagen fiber orientation. TA hydrogel group showed the highest percentage of mixed and horizontal orientation 56% and 36%, respectively. The percentage of horizontal fibers in the TA group is significantly higher than in the negative control group with p-value of 0.0106.

Table 2: Data analysis for collagen fibres orientation

Groups	1=vertical	2=mixed	3=horizontal	P-value
Negative control, no treatment	5 (20%)	20 (80%)	0 (0%)	
Positive control *Hydrocyn® aqua* gel	8 (32%)	13 (52%)	4 (16%)	0.0106
Vehicle hydrogel	7 (28%)	18 (72%)	0%	
Tongkat Ali (TA) hydrogel	2 (8%)	14 (56%)	9 (36%)	

Table 3 shows the results of the pattern of collagen fibers. TA hydrogel group showed the highest results of the 'fascicle' pattern compared to the other groups. The percentage of fascicle pattern in the TA group is significantly higher than the negative control group with a *p-value* of 0.0015.

Table 3: The pattern of collagen fibers; reticular, mixed, and fascicle

Groups	1=reticular	2=mixed	3=fascicle	p-value
Negative control, no treatment	7 (28%)	17 (68%)	1 (4%)	
Positive control *Hydrocyn® aqua* gel	15 (60%)	8 (32%)	2 (8%)	0.001572
Vehicle hydrogel	8 (32%)	17 (68%)	0%	
Tongkat Ali (TA) hydrogel	3 (12%)	14 (56%)	8 (32%)	

Table 4 shows the results for collagen fiber density. TA hydrogel group showed the highest percentage 24% of 'profound' density. The percentage of 'profound' density in the TA group is significantly higher than the negative control group with p= 0.018.

Table 4: Collagen fiber density

Groups	1= minimal	2=moderate	3=profound	p-value
Negative control, no	2 (4%)	23 (96%)	0%	
Positive control *Hydrocyn®*	6 (24%)	19 (76%)	0%	0.018203.
Vehicle hydrogel (Xanthan)	1 (4%)	24 (96%)	0%	
Tongkat Ali (TA) hydrogel	0%	19 (76%)	6 (24%)	

DISCUSSION

Collagen is the fundamental element of the extracellular matrix that plays an essential role in the regeneration of injured skin.[17] Immediately once the skin is injured, the fibroblasts migrate quickly to the wounded site and stimulate the production of new tissue via proliferation.[18] Meanwhile, skin fibroblasts can secrete a wide variety of growth factors and produce collagen.[19] The thickness of the newly generated epidermis and dermis is very important for keeping the healed area. Without the support of a fully developed dermal matrix, the newly formed epidermis is breakable and fragile.[20] Our testing substance showed the thickest epidermal layer with (118.05 μm ± 9.86) compared with the other experimental groups; untreated negative control (67.928 μm ± 34.50), Hydrocyn aqua® gel positive control (90.78 μm ± 41.44) and vehicle (xanthan) hydrogel (113.41 μm ± 2.13), however, the difference was not sizable enough to show statistical significance.

Surprisingly, TA hydrogel and Hydrocyn aqua® gel positive control groups showed a significant increase in the thickness of the newly formed dermis compared with vehicle (xanthan) hydrogel groups with p=0.020 and 0.045 respectively. These results are consistent with its positive effects on wound contraction and re-epithelialisation[16] as well as with its significant effect on VEGF expression in the healing wound.[21] Thus, TA hydrogel is safe and effective to apply on wounded skin as it stimulates collagen production in a comparable way to the reference wound healing agent, Hydrocyn aqua® gel. A relatively thicker epidermis and a significant increase in dermal thickness produced by TA hydrogel indicate the positive effect of topical application of TA hydrogel in the healing process of cutaneous wounds and might point out a superior skin barrier and our testing substance probably better than the others. As collagen is the key element of the dermal layer[22], the significant effect of *Eurycoma longifolia* Jack (TA) hydrogel on dermal thickness (Day 21). Our results are in agreement with the results of Zhang et al.[20] who showed the importance of wound dressing application on epidermal and dermal thickness. The significant effect of *Eurycoma longifolia* Jack (TA) hydrogel on collagen production in the healing wound might be attributed to the availability of phytochemicals with wound healing activity such as alkaloids, flavonoids, glycosides, terpenes, 5-HMF, oleic acid, and palmitic acids.[6,23]

Many previous studies have proved that those phytochemicals stimulate collagen production and cell proliferation through antioxidant and anti-inflammatory properties.[7,8,24] This study also investigated the effect of the topical application of *Eurycoma longifolia* Jack (TA)

hydrogel on wound healing in terms of collagen fibre organisation or orientation.[25] The evaluation of collagen fibres orientation in the excisional wound model in skin rats was evaluated Semi-quantitively according to the score (Vertical = 1, Mixed=2, Horizontal=3).[26] TA hydrogel and Hydrocyn aqua® gel (+ve) control groups showed a significant increase in mixed-oriented collagen fibres in healed skin on Day 21 with 78% and 82% of the histological area investigated respectively compared with the untreated negative control (50%) and vehicle (xanthan) hydrogel (40%) groups.

The organisation of collagen fibres in the healed wound is very important for the strength of the healed wound as the collagen fibres arrangement in the scar differs from that in the dermis. Normal skin collagen fibres are arranged in a basket weave pattern with fibres oriented parallel and perpendicular to the surface, whereas the collagen fibres of weak scars tend to be arranged vertically to the skin surface [22,27] The type of collagen bundles being reticular or fascicular is also of importance to the wound strength.[28] It has been found that fascicular type of collagen bundles give greater strength to the scar in the healed wound.[29] Thus, according to the results, TA hydrogel application leads to the deposition of mixed and horizontal fibers with a minimal percentage of vertical fibers and the highest percentage of fascicular bundles that the other groups. The density of collagen fibers was significantly higher in the TA hydrogel group than in other groups with 24% of the observed fields showing 'profound' collagen density. This finding is a very important indicator that TA hydrogel application significantly increased collagen production. The current study is the first one that investigated the possible effects of *Eurycoma longifolia* Jack (TA) hydrogel in improving the quality of the healed skin in an excisional wound model in rats. Our results confirmed the positive effect of TA hydrogel on collagen production and its quality. The availability of phytochemicals in the root of *Eurycoma longifolia* Jack (TA) with their antioxidant and anti -inflammatory properties could be the main responsible for its efficiency in improving the quality of the healed skin.[30,31,32] Therefore, TA hydrogel can be considered an affordable and effective wound-healing agent that gives better outcomes for healed wounds

CONCLUSION

Topical application of *Eurycoma longifolia* Jack (TA) hydrogel improved the quality of the healed skin by significantly stimulating collagen production by increasing the epidermal and dermal thicknesses of the healed skin and improving the organisation of deposited collagen fibres in a pattern similar to normal skin. *Eurycoma longifolia* Jack (TA) hydrogel could be an effective, affordable, and safe wound healing agent for improving the quality of the healed skin.

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REFERENCES

1. 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. Frontiers in Pharmacology 2018; 21(9):945.
2. Ali A, Garg P, Goyal R, Kaur G, Li X, Negi P, Valis M, Kuca K, Kulshrestha S. A novel herbal hydrogel formulation of moringa oleifera for wound healing. Plants 2020;10(1):25.
3. Shedoeva A, Leavesley D, Upton Z, Fan C. Wound healing and the use of medicinal plants. Evidence-Based Complementary and Alternative Medicine 2019 Sep 22;2019.
4. Sandy-Hodgetts K, Carville K, Leslie GD. Determining risk factors for surgical wound dehiscence: a literature review. International wound journal 2015;12(3):265-75.
5. Umar NM, Parumasivam T, Toh SM. An overview of cutaneous wounds and the beneficial roles of medicinal plants in promoting wound healing. Pharmaceutical Sciences 2021;27(4):489-502..
6. Bektas N, Şenel B, Yenilmez E, Özatik O, Arslan R. Evaluation of wound healing effect of chitosan-based

gel formulation containing vitexin. Saudi Pharmaceutical Journal 2020;28(1):87-94.
7. Sharma A, Khanna S, Kaur G, Singh I. Medicinal plants and their components for wound healing applications. Future Journal of Pharmaceutical Sciences 2021;7(1):1-3.
8. Demilew W, Adinew GM, Asrade S. Evaluation of the Wound Healing Activity of the Crude Extract of Leaves of Acanthus polystachyus Delile (Acanthaceae). Evidence-Based Complementary and Alternative Medicine. 2018 Jun 11;2018:1–9.
9. Liang J, Cui L, Li J, Guan S, Zhang K, Li J. Aloe vera: a medicinal plant used in skin wound healing. Tissue Engineering Part B: Reviews 2021;27(5):455-74.
10. Ruan J, Li Z, Zhang Y, Chen Y, Liu M, Han L, Zhang Y, Wang T. Bioactive constituents from the roots of Eurycoma longifolia. Molecules 2019;24 (17):3157.
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.
12. Alloha IB, Aziz NA, Faisal GG, Abllah Z, Arzmi MH. Effects of Eurycoma Longifolia Jack (Tongkat Ali) alcoholic root extract against oral pathogens. Pharmacognosy Journal. 2019;11(6).
13. Faisal GG, Zakaria SM, Najmuldeen GF. In vitro antibacterial activity of Eurycoma longifolia Jack (Tongkat ali) root extract. IIUM Medical Journal Malaysia. 2015;14(1).
14. Faisal GG, Zakaria SM, Najmuldeen GF, Al-Ani IM. Antifungal activity of eurycoma longifolia jack (tongkat ali) root extract. Journal of International Dental and Medical Research. 2016;9(1):70.
15. Yaseen MR, Faisal GG, Abd Fuaat A, Affandi KA, Alallam B, Nasir MH. Preparation of Euyrycoma Longifolia Jack (EL) Tongkat Ali (Ta) Root Extract Hydrogel for Wound Application. Pharmacognosy Journal 2021;13(6).
16. Al-Bayati MR, Faisal GG, Abd Fuaat A, Affandi KA, Abidin MA. The Effect of Eurycoma longifolia Jack Tongkat Ali Hydrogel on Wound Contraction and Re-Epithelialization in In Vivo Excisional Wound Model. Open Access Macedonian Journal of Medical Sciences. 2022;10(A):634-43.
17. Li CW, Wang Q, Li J, Hu M, Shi SJ, Li ZW, Wu GL, Cui HH, Li YY, Zhang Q, Yu XH. Silver nanoparticles/chitosan oligosaccharide/poly (vinyl alcohol) nanofiber promotes wound healing by activating TGFβ1/Smad signaling pathway. International Journal of Nanomedicine 2016;11:373.
18. Akita S. Wound repair and regeneration: mechanisms, signaling. International Journal of Molecular Sciences 2019;20(24):6328.
19. Zhang X, Xu R, Hu X, Luo G, Wu J, He W. A systematic and quantitative method for wound-dressin 1. Amin ZA, Ali HM, Alshawsh MA, Darvish PH, Abdulla MA. Application of Antrodia camphorata Promotes Rat's Wound Healing In Vivo and Facilitates Fibroblast Cell Proliferation In Vitro [Internet]. Evidence-Based Complementary and Alternative Medicine. 2015. Available from: https://www.hindawi.com/journals/ecam/2015/317693/evaluation. Burns & Trauma 2015 Dec 1;3.
20. Al-Bayati MR, Faisal GG, Abd Fuaat A, Affandi KA, Alallam B. The Effect of the Topical Application of Eurycoma Longifolia Jack (TA) Root Extract Hydrogel on Vascular Endothelial Growth Factor (VEGF) Expression during Wound Healing in vivo Excisional Wound Model. IIUM Medical Journal Malaysia. 2023;22(1).
21. Mathew-Steiner SS, Roy S, Sen CK. Collagen in wound healing. Bioengineering 2021;8(5):63.
22. Kong F, Fan C, Yang Y, Lee BH, Wei K. 5-hydroxymethylfurfural-embedded poly (vinyl alcohol)/sodium alginate hybrid hydrogels accelerate wound healing. International journal of biological macromolecules 2019;138:933-49.
23. Budovsky A, Yarmolinsky L, Ben-Shabat S. Effect of medicinal plants on wound healing. Wound Repair and Regeneration 2015;23(2):171-83.
24. Ehrlich HP, Krummel TM. Regulation of wound healing from a connective tissue perspective. Wound repair and regeneration 1996;4(2):203-10.
25. Gupta A, Kumar P. Assessment of the histological state of the healing wound. Plastic and Aesthetic Research 2015;2:239-42.
26. Clemons TD, Bradshaw M, Toshniwal P, Chaudhari

N, Stevenson AW, Lynch J, Fear MW, Wood FM, Iyer KS. Coherency image analysis to quantify collagen architecture: implications in scar assessment. RSC advances 2018;8(18):9661-9.
27. Magnusson SP, Heinemeier KM, Kjaer M. Collagen homeostasis and metabolism. Metabolic influences on risk For tendon disorders 2016:11-25.
28. Silver FH, Jaffe M, Shah RG. Structure and behavior of collagen fibers. InHandbook of properties of textile and technical fibres 2018 (pp. 345-365). Woodhead Publishing.
29. Abubakar BM, Salleh FM, Wagiran A. Chemical composition of Eurycoma longifolia (Tongkat Ali) and the quality control of its herbal medicinal products. Journal of Applied Sciences 2017;17(7):324-38.
30. Khanam Z, Wen CS, Bhat IU. Phytochemical screening and antimicrobial activity of root and stem extracts of wild Eurycoma longifolia Jack (Tongkat Ali). Journal of King Saud University-Science 201;27(1):23-30.
31. Zizzo MG, Caldara G, Bellanca A, Nuzzo D, Di Carlo M, Serio R. Preventive effects of guanosine on intestinal inflammation in 2, 4-dinitrobenzene sulfonic acid (DNBS)-induced colitis in rats. Inflammopharmacology 2019;27:349-59.