

The wound healing potential of 2,3 dimethylquinoxalinehydrogel in rat excisional wound model.

Abstract

Background: 2,3-dimethylquinoxaline (DMQ) is a naturally occurring compound with documented antifungal activity. It showed also good in vitro physicochemical and pharmacokinetic characteristics.

Methods. The wound healing activity of 1% DMQ hydrogel in healthy adult male Wistar rats were evaluated using the excision wound model. On day 7, the mean percentage closure of the wound area was determined. The animals were sacrificed on day 7 and skin was isolated for histology research and assessment of some inflammatory and oxidative markers, hydroxyproline and tissue growth factor.

Results and discussion Almost complete wound healing was observed after treatment with DMQ 1 % Jell for 7 days. The histological study confirmed marked attenuation of wound-induced histological changes. There was a marked reduction in TNF- α , IL-6, IL- β 1 and NF- κ B. levels. These data suggest the potential anti-inflammatory effect of DMQ.

Conclusion DMQ has potential skin wound healing ability likely due to its anti-inflammatory mechanism. Further study is needed to confirm these preliminary findings and explore the molecular mechanism

Keywords: 2,3-dimethylquinoxaline, hydrogel, wound healing, inflammatory biomarkers.

Formatted: Font: Not Bold

Introduction

Wounds are defined as harm to the skin's structural integrity brought on by many causes such as burns, cuts, scalds, and lesions such as foot complications of diabetes.(Young and McNaught, 2011). Improper management of wounds is associated with many complications such as infections, wound dehiscence, inflammation, scarring, and improper angiogenesis and regeneration.(Chadwick et al., 2012, Aldridge, 2015).

It is anticipated that chronic wounds will continue to be a significant challenge because of the ageing population, and the ongoing increase in diabetes, obesity, and infections around the globe.(Sen, 2021). These complications increase morbidity and mortality, negatively impact patients' quality of life, and place a tremendous financial load on healthcare systems. Different approaches are used to promote wound healing, including cell therapy, gene therapy, growth factor delivery, wound dressings, and skin grafts. However, none of these modalities is effective for all kinds of wounds(Kolimi et al., 2022). Moreover, most sophisticated approaches are expensive and not readily available in developing countries. As a result, it is essential to create newer and creative therapy methods for multifaceted therapeutic regimens for chronic wounds.

The use of herbal natural products as possible helpful agents in the process of wound healing has been the subject of an increasing number of research papers.(Ramalingam et al., 2022). Indeed, the main benefits of using these herbal remedies are their low cost, availability, and minimal side effects. In this context, the efficacy of phytochemicals in the management of wounds is attributed to their antimicrobial, anti-inflammatory, and antioxidant effects. Some phytochemicals also influence the mitogen-activated protein kinase (MAPK) and transforming growth factor-beta (TGF-) signaling pathways.(Shah and Amini-Nik, 2017, Thangapazham et al., 2016, Binsuwaidan et al., 2022, Criollo-Mendoza et al., 2023, Wan et al., 2022). Examples of extensively studied phytochemicals for potential wound healing include flavonoids (Zulkefli et al., 2023). The wound-healing activity of other active ingredients such as alkaloids, saponins, and terpenoids was also reported (Balkrishna et al., 2022).

Some quinoxaline derivatives reported to possess wound-healing potential (Geefhavani et al., 2012). Quinoxaline derivatives were patented for anticancer and antimicrobial activity (González and Cerecetto, 2012). Charles and Minakiri reported the natural presence of 2,3-dimethylquinoxaline (DMQ) (Fig. 1) in the *Chromolaena odorata* plant(Charles and Minakiri, 2018). They suggested that the observed antimicrobial activity of *Chromolaena odorata* could be

attributed to the presence of DMQ. Alfadil and his colleagues confirmed that DMQ had a broad-spectrum antifungal activity (Alfadil et al., 2021).

The lipophilicity of DMQ was determined by the distribution coefficient ($\log D_{7.4}$) between n-Octanol and phosphate buffer at pH 7.4. It was significantly ($P < 0.05$) higher than tolbutamide and lower than ketoconazole. DMQ had a significant ($P < 0.05$) high permeability compared to antipyrine, atenolol, talinolol, and estrone 3-sulfate. It showed a good safety profile in both in vitro and animal toxicity studies (Alsamhan, 2022).

Therefore, the present pilot study aims to explore the potential efficacy of DMQ for accelerating skin wound healing.

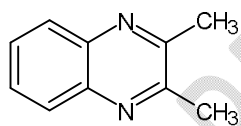


Fig 1: [Structure of 2,3-dimethylquinoxaline](#)

2. Materials and Methods

Materials

2,3-Dimethylquinoxaline (DMQ), (Quinoxaline-2,3-dimethyl) (Cat. No. D184977; Chemical Abstracts Service, CAS #2379-55-7), Sigma. Hydroxypropyl methylcellulose (HPMC) and Methanol were pharmaceutical grades.

Methods

Preparation of DMQ hydrogel

The composition of plain HPMC (2% w/w) 100 g Jell is 2 gm HPMC and distilled water 100 gm. DMQ is soluble in methanol (100 mg/mL). The plain jell was prepared as follows: 2 g of the polymer was weighed and placed in a suitable beaker, 98 g of water was added to it, the container was covered and left for 24 hours to form the gel.

Formulation of the medicated Jell. 1% w/w

0.5 gram of DMQ was dissolved in 4.5 g of methanol (final concentration 100 mg/g)

The drug solution in methanol was added gradually to 45 grams of the plain HPMC jell, with continued gentle mixing. Both plain and medicated jell were stored in suitable jars and stored at room temperature. The stability of DMQ was insured by spectrophotometric analysis.

Animal study

Male Wistar rats weighing about 200 g were used. Animals were kept at a relative humidity range of 40–70% and a temperature of 22 ± 2 °C, under a 12/12 h light–dark cycle. The animal handling procedures were approved by the Committee of Research Ethics, Faculty of Pharmacy, KAU.

Skin Irritation Test

This experiment was previously done in a previously published study.

Wound Excision

The rodents were sedated with ketamine (90 mg/kg). To disinfect their shaved skin on the dorsal area, 75% ethanol was used. This was followed by the excision of a 100 mm² region of skin on the rat's dorsal surface. After disinfecting the wounds, the pain was reduced by injecting 4 mg/kg of lidocaine hydrochloride (2%) with 1:80,000 epinephrine (SC).

Each rat was put in its cage and given standard feed pellets and water ad libitum. The rats were split into three groups at random as follows: Group 1: no procedure, control (n=2); Group 2: treated daily with plain hydrogel (vehicle n=3); and Group 3: treated daily DMQ hydrogel (n=3). Treatment was continued for 7 days. On days 0 & 7, wound diameters (WD) were measured, and lesions were photographed. On day 7, one randomly selected rat from each group was decapitated, and the wounded skin was dissected. A portion of the skin was preserved in 10% neutral formalin for histology study. The other portion was thoroughly rinsed with ice-cold saline then immediately frozen in liquid nitrogen and stored at -80 °C for analysis of biochemical and immunological and other biomarkers in skin tissue homogenate.

Wound Measurement

The percentage of wound contraction was determined according to the following formula:

$$\text{Wound contraction \%} = (\text{WD on day 0} - \text{WD on day 7 or 14}) / (\text{WD on day 0}) \times 100$$

Preparation of Tissue Homogenate

The frozen skin specimens were delicately blotted between filter papers and weighed. Then 10% (w/v) homogenates were prepared in phosphate-buffered saline (PBS, 50 mM potassium

phosphate, pH 7.4, ice-cooled) before spinning at 3000 rpm for 20 minutes at 4 °C. Using the supernatant, specific parameters were estimated.

Biochemical Analyses

The following biochemical parameters (oxidative stress) were assessed in skin tissue homogenate. Assessments of the total protein, CAT, SOD, GSH, and MDA were performed using biochemical ELISA kits (Cayman[®] Chemical, Ann Arbor, MI, USA). based on the instructions provided by the manufacturer.

Analyses of pre-inflammatory markers

The following pre-inflammatory markers were assessed in skin tissue homogenate using ELISA Kits: TNF- α , IL-6, IL- β 1 and NF- κ B. levels. based on the instructions provided by the manufacturer

Histological Examination

Skin tissues were preserved in a 10% neutral formalin solution for 24 hours before being dehydrated, embedded in paraffin, and then sectioned in a paraffin block (5 μ m thickness). Following dewaxing, tissues were rehydrated, stained with hematoxylin and eosin (H&E),

Results

Wound contraction % was about 97 % after 7 days after treatment with DMQ 1 % Jell compared to about 84 % in the case of the plain hydrogel.

Macroscopic wound closure at 0, 7 days after treatment with plain and DMQ 1 % Jell is presented in fig 2. Almost complete wound healing was observed after treatment with DMQ 1 % Jell. Treatment with plain hydrogel was associated with a reduction in wound size but incomplete healing.

Micrographs of H&E-stained wounded tissues of Wistar rats are presented in figure 3. The histological study confirmed that treatment with DMQ 1 % Jell for 7 days led to marked attenuation of wound-induced histological changes of the skin and documented as the resolution of neutrophil infiltration that was still observed in the skin of rats treated with plain jell

A panel of biochemical markers of oxidative stress markers in skin tissue homogenate is presented in table 1. These data show a slight decrease in GSH and an increase in SOD levels after treatment with DMQ 1 % Jell but the level of CAT & MDA are similar to those after treatment with plain hydrogel.

A panel of inflammatory markers in skin tissue homogenate is presented in Table 2: There was a marked reduction of the level of all tested parameters (TNF- α , IL-6, IL- β 1 and NF- κ B.)

after treatment with DMQ 1 % Jell, These data suggest a potential anti-inflammatory effect of DMQ.

Hydroxyproline and TGFB1 in skin tissue homogenate are presented in Table 3. The level of both Hydroxyproline and TGFB1 was increased after treatment with DMQ 1 % Jell suggesting an acceleration of wound proliferation.





	D0	D7
Plain jell		
DMQ 1% Jell		

Fig 2: Wound healing evaluation in Wistar albino rodents with complete thickness excision wounds. Macroscopic wound closure at 0, 7 days after treatment with plain and DMQ 1 % Jell

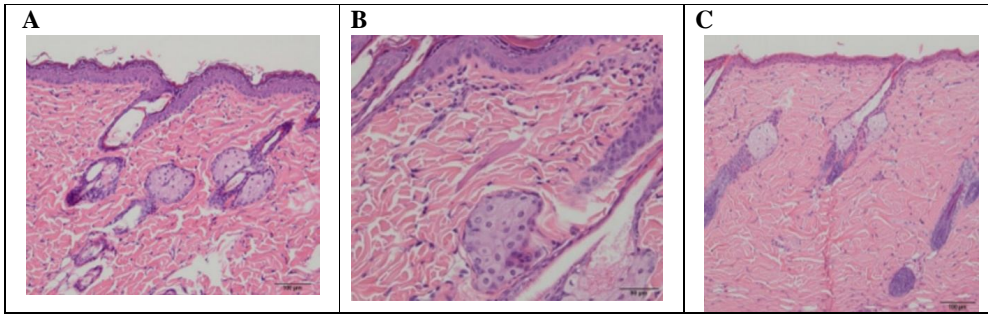


Fig 3: Micrographs of H&E-stained wounded tissues of Wister rats
 A: control, B plain jell treated, C: 1 % DMQ Jell treated after 7 days of treatment

Table 1: Panel of ~~Biochemical~~ biochemical markers of oxidative stress markers in skin tissue homogenate

groups	SOD	CAT	GSH	MDA
	u/ml	u/ml	ng/ml	nmol/ml
DMQ 1%	68	122	2.9	2.00
Plain jell	80	121	4.0	1.98

Table 2: Panel of inflammatory markers in skin tissue homogenate

groups	TNF-a	IL-6	IL-B1	NF-Kb
	pg/ml	pg/ml	pg/ml	ng/ml
DMQ 1%	56	16.3	38	44
Plain jell	82	18	56.2	67

Table 3: Hydroxyproline and TGFB1 in skin tissue homogenate in skin tissue homogenate

groups	hydroxyproline	TGFB1
	pg/ml	pg/ml
DMQ 1%	1.5	10.4
Plain jell	0.94	7.8

Discussion

Wound healing is an optimized biological mechanism that occurs in a series of contiguous stages that include hemostasis, inflammatory scenarios, the proliferation of cells, and tissue remodeling (Wallace et al., 2017, Goldberg and Diegelmann, 2017).

The present pilot study suggested the potential healing properties of topically applied DMQ. The exact mechanism needs more detailed studies. However, currently, limited data suggest that its anti-inflammatory effect is likely involved in its obvious ability to accelerate skin wound healing in healthy rats.

It has been established that reactive oxygen species (ROS) play an important part in several stages of healing. ROS are fundamental to all wound healing processes because low levels of ROS production are required for the fight against invading microorganisms and cell survival signaling. However, excessive ROS generation or impaired ROS detoxification causes oxidative damage, which is the primary cause of chronic wounds that do not resolve. In this context, experimental and clinical research has shown that antioxidant and anti-inflammatory strategies can help to speed up wound healing (Cano Sanchez et al., 2018). As a result, various antioxidant approaches help improve wound healing (Fitzmaurice et al., 2011, Comino-Sanz et al., 2021, Xu et al., 2020).

Inflammation is a physiological process in the healing process (Bryan et al., 2012). However, prolonged amounts of ROS production in the wound can lead to chronic inflammation which unfavourably/unfavorably affects wound healing. The inflammation begins with the neutrophil influx, which is aided by mast cell histamine production. The neutrophils promote the release of pro-inflammatory cytokines (e.g., TNF- α , IL-1, and IL-6), phagocytosis, and protease secretion, which contribute to the attraction of other inflammatory cells, the amplification of the inflammatory response, the death of microbial pathogens, and the stimulation of regenerative and remodelling factors (Rodrigues et al., 2019).

~~Interleukin IL~~ Interleukin IL-6 (a proinflammatory cytokine) is essential for wound healing and plays an important part in acute inflammation. It is released early in reaction to injury and induces the release of proinflammatory cytokines from tissue-resident macrophages,

keratinocytes, endothelial cells, and stromal cells. (Johnson et al., 2020). The present study showed a moderate reduction of IL-6 in the skin tissues of rats treated with DMQ hydrogel

Hydroxyproline, a non-essential amino acid, is necessary for the production of collagen and the thermodynamic equilibrium of collagen's triple-helical conformation and associated tissues. Its low amount indicates poor wound healing-(Kumar Srivastava et al., 2016). Transforming growth factor-beta 1 (TGF- β 1) is a multifunctional cytokine implicated in a variety of human illnesses. It is thought to play an essential role in wound healing and repair because it is a key regulator of extracellular matrix production and remodelling via its impact on mesenchymal cells. (Varga et al., 1987, Pakyari et al., 2013, Klass et al., 2009). In the present pilot study, an increase in tissue content of Hydroxyproline, and TGF B1 was noticed after treatment with DMQ hydrogel.

Several pharmacological approaches for the management of wounds target the inflammatory response (Shukla et al., 2019). Certain phytochemicals and natural products can modulate inflammation through a variety of pathways, including the regulation of growth factors and cytokines. Examples of topical application of natural compounds for enhancing wound healing include aloe vera (Hashemi et al., 2015, Hekmatpou et al., 2019), It has a broad spectrum of effects that synergistically enhance wound healing. One of these effects is its capacity to suppress inflammatory responses by inhibiting IL-6 and IL-8, reducing leukocyte adhesion, increasing IL-10 levels, and decreasing TNF- α levels-(Mosayebi et al., 2009). Another example is curcumin which works by decreasing TNF- α , IL-1, and MMP-9 expression while increasing anti-inflammatory cytokine IL-10 and antioxidant enzymes at the lesion site-(Barchitta et al., 2019).

Conclusion-

DMQ has potential skin wound healing ability likely due to its anti-inflammatory mechanism. Further study is needed to confirm these preliminary findings and to explore the molecular mechanism.

UNDER PEER REVIEW

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 (The Biomedical Ethics Committee approved the experimental protocol at King Abdulaziz University, KAU and the National Committee of Bioethics, NCBE, Registration No (PH-1443-76).

All experiments have been examined and approved by the appropriate ethics committee. Animal handling was performed in strict compliance with the ethical guidelines for treating animals as defined by KAU and NCBE

References

- ALDRIDGE, P. 2015. Complications of wound healing: causes and prevention. *Companion Animal*, 20, 453-459.
- ALFADIL, A., ALSAMHAN, H. A., ALI, A. S., ALKREATHY, H. M., ALRABIA, M. W. & ABDULLAH, H. Y. 2021. In vivo and In vitro Antifungal Activity of 2, 3-Dimethylquinoxaline. *Journal of Pharmaceutical Research International*, 198-207.
- ALSAMHAN, H. A. 2022. *Antifungal Activities and Toxicities of Certain Quinoxaline Derivatives in Experimental Animals*. PhD, King Abdulaziz University.
- BALKRISHNA, A., SAKSHI, CHAUHAN, M., DABAS, A. & ARYA, V. 2022. A Comprehensive Insight into the Phytochemical, Pharmacological Potential, and Traditional Medicinal Uses of *Albizia lebbek* (L.) Benth. *Evid Based Complement Alternat Med*, 2022, 5359669.
- BARCITTA, M., MAUGERI, A., FAVARA, G., MAGNANO SAN LIO, R., EVOLA, G., AGODI, A. & BASILE, G. 2019. Nutrition and wound healing: An overview focusing on the beneficial effects of curcumin. *International journal of molecular sciences*, 20, 1119.
- BINSUWAIDAN, R., ELEKHAWY, E., ELSEADY, W. S., KESHK, W. A., SHOEIB, N. A., ATTALLAH, N. G., MOKHTAR, F. A., ABD EL HADI, S. R., AHMED, E. & MAGDELDIN, S. 2022. Antibacterial activity and wound healing potential of *Cycas thouarsii* R. Br n-butanol fraction in diabetic rats supported with phytochemical profiling. *Biomedicine & Pharmacotherapy*, 155, 113763.
- BRYAN, N., AHSWIN, H., SMART, N., BAYON, Y., WOHLERT, S. & HUNT, J. A. 2012. Reactive oxygen species (ROS)—a family of fate-deciding molecules pivotal in constructive inflammation and wound healing. *Eur Cell Mater*, 24, e65.
- CANO SANCHEZ, M., LANCEL, S., BOULANGER, E. & NEVIERE, R. 2018. Targeting oxidative stress and mitochondrial dysfunction in the treatment of impaired wound healing: a systematic review. *Antioxidants*, 7, 98.
- CHADWICK, S., HEATH, R. & SHAH, M. 2012. Abnormal pigmentation within cutaneous scars: A complication of wound healing. *Indian Journal of Plastic Surgery*, 45, 403-411.
- CHARLES, J. I. & MINAKIRI, I. S. 2018. Evaluation of the Antibacterial Activity of *Chromolaena Odorata* in Wistar Rats and its Chemical Characterization. *American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS)*, 42, 111-129.
- COMINO-SANZ, I. M., LÓPEZ-FRANCO, M. D., CASTRO, B. & PANCORBO-HIDALGO, P. L. 2021. The role of antioxidants on wound healing: A review of the current evidence. *Journal of Clinical Medicine*, 10, 3558.

- CRIOLLO-MENDOZA, M. S., CONTRERAS-ANGULO, L. A., LEYVA-LÓPEZ, N., GUTIÉRREZ-GRIJALVA, E. P., JIMÉNEZ-ORTEGA, L. A. & HEREDIA, J. B. 2023. Wound Healing Properties of Natural Products: Mechanisms of Action. *Molecules*, 28.
- FITZMAURICE, S., SIVAMANI, R. & ISSEROFF, R. 2011. Antioxidant therapies for wound healing: a clinical guide to currently commercially available products. *Skin pharmacology and physiology*, 24, 113-126.
- GEEFHAVANI, M., REDDY, J. & SATHYANARAYANA, S. 2012. Synthesis, antimicrobial and wound healing activities of diphenyl quinoxaline derivatives. *International Journal of Pharmacy and Technology*, 4, 4700-4710.
- GOLDBERG, S. R. & DIEGELMANN, R. F. 2017. The basic science of wound healing. *Critical Limb Ischemia: Acute and Chronic*, 131-136.
- GONZÁLEZ, M. & CERECETTO, H. 2012. Quinoxaline derivatives: a patent review (2006--present). *Expert Opin Ther Pat*, 22, 1289-302.
- HASHEMI, S. A., MADANI, S. A. & ABEDIANKENARI, S. 2015. The review on properties of Aloe vera in the healing of cutaneous wounds. *BioMed research international*, 2015.
- HEKMATPOU, D., MEHRABI, F., RAHZANI, K. & AMINIYAN, A. 2019. The Effect of Aloe Vera Clinical Trials on Skin Wound Prevention and Healing: A Systematic Review. *Iran J Med Sci*, 44, 1-9.
- JOHNSON, B. Z., STEVENSON, A. W., PRÊLE, C. M., FEAR, M. W. & WOOD, F. M. 2020. The role of IL-6 in skin fibrosis and cutaneous wound healing. *Biomedicines*, 8, 101.
- KLASS, B., GROBBELAAR, A. & ROLFE, K. 2009. Transforming growth factor β 1 signalling, wound healing and repair: a multifunctional cytokine with clinical implications for wound repair, a delicate balance. *Postgraduate Medical Journal*, 85, 9-14.
- KOLIMI, P., NARALA, S., NYAVANANDI, D., YOUSSEF, A. A. A. & DUDHIPALA, N. 2022. Innovative Treatment Strategies to Accelerate Wound Healing: Trajectory and Recent Advancements. *Cells*, 11, 2439.
- KUMAR SRIVASTAVA, A., KHARE, P., KUMAR NAGAR, H., RAGHUWANSHI, N. & SRIVASTAVA, R. 2016. Hydroxyproline: a potential biochemical marker and its role in the pathogenesis of different diseases. *Current Protein and Peptide Science*, 17, 596-602.
- MOSAYEBI, G., GHAZAVI, A., AGHILI, B. & MIRSHAFIEI, A. 2009. Immunomodulating activity of Aloe Vera in an animal model of multiple sclerosis. *Journal of Arak University of Medical Sciences*, 12, 109-115.
- PAKYARI, M., FARROKHI, A., MAHARLOOEI, M. K. & GHAHARY, A. 2013. Critical Role of Transforming Growth Factor Beta in Different Phases of Wound Healing. *Adv Wound Care (New Rochelle)*, 2, 215-224.
- RAMALINGAM, S., CHANDRASEKAR, M. J. N. & NANJAN, M. J. 2022. Plant-based Natural Products for Wound Healing: A Critical Review. *Curr Drug Res Rev*, 14, 37-60.
- RODRIGUES, M., KOSARIC, N., BONHAM, C. A. & GURTNER, G. C. 2019. Wound healing: a cellular perspective. *Physiological reviews*, 99, 665-706.
- SEN, C. K. 2021. Human Wound and Its Burden: Updated 2020 Compendium of Estimates. *Adv Wound Care (New Rochelle)*, 10, 281-292.
- SHAH, A. & AMINI-NIK, S. 2017. The role of phytochemicals in the inflammatory phase of wound healing. *International journal of molecular sciences*, 18, 1068.
- SHUKLA, S. K., SHARMA, A. K., GUPTA, V. & YASHAVARDDHAN, M. 2019. Pharmacological control of inflammation in wound healing. *Journal of tissue viability*, 28, 218-222.

- THANGAPAZHAM, R. L., SHARAD, S. & MAHESHWARI, R. K. 2016. Phytochemicals in wound healing. *Advances in wound care*, 5, 230-241.
- VARGA, J., ROSENBLOOM, J. & JIMENEZ, S. 1987. Transforming growth factor β (TGF β) causes a persistent increase in steady-state amounts of type I and type III collagen and fibronectin mRNAs in normal human dermal fibroblasts. *Biochemical Journal*, 247, 597-604.
- WALLACE, H. A., BASEHORE, B. M. & ZITO, P. M. 2017. Wound healing phases.
- WAN, X., CHEN, Y., GENG, F., SHENG, Y., WANG, F. & GUO, J. 2022. Narrative review of the mechanism of natural products and scar formation in wound repair. *Ann Transl Med*, 10, 236.
- XU, Z., HAN, S., GU, Z. & WU, J. 2020. Advances and impact of antioxidant hydrogel in chronic wound healing. *Advanced healthcare materials*, 9, 1901502.
- YOUNG, A. & MCNAUGHT, C.-E. 2011. The physiology of wound healing. *Surgery (Oxford)*, 29, 475-479.
- ZULKEFLI, N., CHE ZAHARI, C. N. M., SAYUTI, N. H., KAMARUDIN, A. A., SAAD, N., HAMEZAH, H. S., BUNAWAN, H., BAHARUM, S. N., MEDIANI, A., AHMED, Q. U., ISMAIL, A. F. H. & SARIAN, M. N. 2023. Flavonoids as Potential Wound-Healing Molecules: Emphasis on Pathways Perspective. *Int J Mol Sci*, 24.