2024, Scienceline Publication
World's Veterinary Journal
World Vet J, 14(3): 311-317.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2024.wvj37
PII: S232245682400037-14

Effect of *Aloe vera* Gel and Sodium Metabisulphite on Expression of Fibroblast Growth Factor in Incision Wound of Rats

Yulia Wiji Purnamasari[1], Juniarti[2*], Nunung Ainur Rahmah[2], Hafid Ernanda[1], and Wening Sari[2]

[1]*Biomedical Sciences Department, Universitas Yarsi, Jakarta, Indonesia*
[2]*Department of Pharmacology, School of Medicine, Universitas Yarsi, Jakarta, Indonesia*

*Corresponding's author's Email: jiezka1701@gmail.com

ORIGINAL ARTICLE
Received: June 24, 2024
Revised: July 20, 2024
Accepted: August 26, 2024
Published: September 25, 2024

ABSTRACT

An incision wound is a wound caused by being sliced. Two ingredients that play a key role in the wound-healing process are glucomannan and acemannan, which are rich in polysaccharides and growth hormones. Growth hormones stimulate fibroblast activity and proliferation. The present study involved 35 Sprague Dawley male rats, aged 2-3 months old and weighing 200-300 grams. The study comprised seven groups including, negative control group (G1), positive control (aquades, G2), betadine 10% (G3), gel base (0.5 mg, G4), gel base + sodium metabisulfite 0.2 gr (G5), gel base + *Aloe vera* 5% (G6), and gel base + *Aloe vera* 5% + sodium metabisulfite 0.2 gr (G7). Each group had five replications. Initially, a 4-cm incision was made on the dorsal skin of each rat. The study lasted 15 days with observations made on days 3, 7, and 15. After the observation period, the rats were anesthetized and then terminated to collect skin tissues for microscopic examination. The tissue samples were then stained immunohistochemically to assess fibroblast growth factor (FGF) expressions. The results showed that the highest FGF expression was observed in the 5% *Aloe vera* + 2% metabisulfite group (G7), while the lowest FGF expression was in the negative control group (G1). It is concluded that *Aloe vera* L. extract gel at 5% + 2% metabisulfite (G7) significantly enhances the expression of FGF.

Keywords: *Aloe vera* L, Fibroblast growth factor, Incision wound, Skin, Sodium metabisulfite

INTRODUCTION

An incision wound is an injury caused by a sharp object slicing the skin. The wound healing process consists of four different phases including homeostasis, inflammation, proliferation, and maturation (LaiCheong and McGrath, 2017). The migration and proliferation of fibroblasts in the wound area play a crucial role in this process. An increase in fibroblast cells affects the amount of collagen fibers, which can be linked to the edges of wounds, form connective tissue, and provide strength and integrity to the wound.

Conventional Treatments for incision wounds typically include povidone-iodine and topical antibiotics (Wilantari, 2020). However, povidone Iodine 10% has several side effects such as bacterial resistance and hypersensitivity, inhibiting the wound granulation process (Amiruddin et al., 2015). To mitigate these drawbacks, herbal remedies such as *Aloe vera* can be recommended as an alternative and complementary treatment.

Aloe vera L. is a plant that contains materials for wound healing. According to Nurhidayanti (2022), *Aloe vera* has several vitamins, minerals, enzymes, polysaccharides, glucomannans, acemannans, polypakaride compounds, and organic acids that are water-soluble and fat-soluble. Glucomannans and acemannas plays an important role in the wound healing phase, as they activate macrophages, which play a key role in regulating tissue repair. Macrophages release cytokines and growth factors (PDGF, TGF-a, TGF-b, EGF VEGF) that facilitate tissue repair. Additionally, glucomannas and acemannas activate and proliferate fibroblasts, accelerating the expression of vascular endothelial growth factor (VEGF), which stimulates the formation of new capillaries in the wound-healing process. *Aloe vera* gel also contains lignin, which can penetrate and absorb into the skin, playing a significant role in the proliferation phase by promoting new cells and preventing excess fluid loss from the skin surface (Hekmatpou et al., 2019). Mustaqim et al. (2018) reported that the administration of *Aloe vera* gel on mouse wounds improved wound healing, as evidenced by the parameters of epithelial thickness and the average number of fibroblasts.

Sodium metabisulfite is a preservative substance. It is used as an antioxidant and antimicrobial agent which functions as a preservative in foodstuff, syrup medicines, cosmetic preparations, and hair nourishers (Ilie-mihai et al.,

To cite this paper: Purnamasari YW, Juniarti, Rahmah NA, Ernanda H, and Sari W (2024). Effect of *Aloe vera* Gel and Sodium Metabisulphite on Expression of Fibroblast Growth Factor in Incision Wound of Rats. *World Vet. J.,* 14(3): 311-317. DOI: https://dx.doi.org/10.54203/scil.2024.wvj37

2022). In the process of healing incisional wounds, sodium metabisulfite functions as an antimicrobial that inhibits bacterial contamination of skin tissue. As a preservative, it also helps maintain the stability of the *Aloe vera* content in the preparation. The present study aimed to evaluate the effect of the formulation of *Aloe vera* extract gel with the addition of sodium metabisulfite preservatives on the expression of FGF in wound healing.

MATERIALS AND METHODS

Ethical approval

The animal experimentation was approved by the Ethical Clearance Committee of YARSI University Research Institute, Jakarta, with registration number 135/KEP-UY/EA.10/VI/2023. This study was conducted from July to December 2023 at the Animal Research Facility, Jakarta, Indonesia.

Study design

The manufacture of the gel base was carried out at the Pharmacy Laboratory of PT., Derma Beauty Indonesia, Tangerang. The *Aloe vera* samples were washed, and the skin of the leaf gel was separated, cut into small pieces, and macerated overnight in ethanol solvent. The filtrate was separated from the residue and then evaporated using a rotary evaporator (Japan) under vacuum pressure at a temperature of ± 40°C to obtain an ethanol extract (Baldi et al., 2021).

Gel base production

The tools used included a homogenizer, a beaker, a gel placement container, and a digital scale. There was a 5% extract of *Aloe vera* L in 100-gram preparations. The gel base was prepared using *Aloe vera* extract, where 5% of the extract was calculated to be added. This means that after adding 5 g of extract to 100 grams of the gel base, the resulting amount of the gel base was 95 grams. This mixture was homogenized using a Thinky mixer (USA), at a speed of 2000 rpm for 3 minutes until homogeneous. Sodium metabisulfite (Indonesia) was added at a rate of 0.2 grams and mixed at the final stage. Next, the gel base was mixed with *Aloe vera* L extract and sodium metabisulfite at a predetermined dose using a mixer at a speed of 2000 rpm for 3 minutes. The gel base was then evaluated organoleptically for its physical stability, acidity, viscosity, and total plate and yeast mold counts for 8 weeks (Retnowati et al., 2021).

The study used male Sprague Dawley rats, 2-3 months old, weighing 200-300 grams. The rats were divided into seven groups with five repetition rats in each group including, Group 1 (negative control), Group 2 (positive control given aquades 0.5 ml), Group 3 (positive control given betadine 10 %, 0.5 ml, Indonesia), Group 4 group (given the gel base), Group 5 (given the gel base and sodium metabisulfite 0.2 gr), Group 6 (given the gel base and *Aloe vera* 5%), and Group 7 (given *Aloe vera* 5%, and sodium metabisulfite 0.2 gr). Treatment for all groups was conducted over 15 days. To begin, the rats were given anesthesia with ketamine (10 mg/kg BW) and xylazine (2 mg/kg BW, Sotoudeh and Namavar, 2022) intraperitoneally. After anesthesia, the fur on the rats' backs was shaved to a size of 5 x 3 cm. The rats were then placed on the operating table and fixed according to the procedure (Kartika et al., 2013). The skin was disinfected with betadin, rubbed with 70% alcohol, and then a 4cm long incision was made on the back. Observations were made on days 3, 7, and 15. At the end of the observation period, the rats were anesthetized and terminated using cervical dislocation. Finally, the skin tissue was taken for microscopic examination.

Sample collection

The rats were maintained for 15 days. On day 15, the skin samples were collected and fixed in 10% neutral buffer formalin.

Laboratory test

The skin samples were dehydrated using graded alcohol and xylene and then blocked using liquid paraffin (Alturkistani et al., 2015). The samples were cut using a microtome. The skin sections were then stained by immunohistochemia (IHC) antibody for fibroblast growth factor (Haid et al., 2020). The slides were then dehydrated and treated with endogenous peroxidase and protein block. Next, they were incubated using antibody primer FGF2 (Vantec bio) for several minutes. Following this, the slides were incubated with a post-primary antibody and diethylaminobenzidine (DAB). They were finally photographed using an Olympus microscope (CX33, Japan) at 400 × magnification and analyzed using ImageJ software (NIH, USA).

Data analysis

Data were analyzed quantitatively using SPSS version 25. The analysis was conducted using the Kruskal Wallis non-parametric test followed by the Mann-Whitney post hoc test. Correlation analysis was performed using the Spearman test with a confidence level of 95% ($p < 0.05$).

RESULTS AND DISCUSSION

The results of the observation of organoleptic tests on the formulation of gel preparations on day 1 did not show any changes in color, odor, or texture. Organoleptic tests (Table 1 and Figure 1a) on the formulation of gel preparations for 8 weeks indicated no changes in the texture, color, and odor of the preparations. This stability is because of the presence of sodium metabisulfite. The addition of sodium metabisulfite was proven to increase the shelf life of the gel-based *Aloe vera* L. extraction formulation. This is due to the role of sodium metabisulfite as a preservative when added to drug formulations because sodium metabisulfite has the function of preventing the growth of microbes (Yusuf et al., 2020). The acidity test in the gel formulation of *Aloe vera* extract shown a pH of 5.1 (Table 1 and Figure 1b). This indicates that preparations have the same pH value of 4.5-6.5 as human skin (Yusuf et al., 2020). The viscosity test on the formulation of gel preparations showed a viscosity value of 5420 centipoise (cps) (Table 1 and Figure 1c). This value indicates that preparations had a low viscosity (5640 cps). Mixing sodium metabisulfite may dilute the formulation, as the small molecular weights of sodium metabisulfite and *Aloe vera* cause a decrease in the overall consistency of the preparation. However, the viscosity results for the preparations remain within the normal range because they are still in the range of 2000–50000 cps (Yusuf et al., 2020). The test of the total plate count and yeast mold count showed that the formulations had low microbial counts (Table 1 and Figure 1d). This finding proves that the addition of sodium metabisulfite to formulations can prevent the development of microbes in the preparations. This is in accordance with research by Kristantri et al. (2022), who reported that microbial agents developed more quickly in preparations without sodium metabisulfite. The reason is that sodium metabisulfite, apart from being an antioxidant, also acts as an inhibitor of microbial agents in preparations (Table 1).

In this study, the results indicated a significant increase in FGF expression after treatment with *Aloe vera* L extract gel and sodium metabisulfite ($p < 0.05$). The increase in FGF expression on day 7 was higher than on day 3. The highest FGF expression intensity was observed on days third and seventh, in the *Aloe vera* + sodium metabisulfite group compared to other groups. In this study, FGF expression increased on days 3 and 7 but decreased on day 15 (Figure 2).

Table 1. Gel stability test (pH, viscosity, total plate count, and yeast mold count)

Test	Results
Acidity degree (pH)	5.1
Viscosity	5420 cps
Total plate count	< 10^1 cfu/g
Yeast mold count	1×< 10^cfu/g

Description: Stability test results of 5% *Aloe vera* gel + 0.2 gr of sodium metabisulfite

Figure 1. Stability test results of gel preparations. **a:** Organoleptic test, **b:** pH test, **c:** Viscosity test, and **d:** Total plate test and yeast mold test

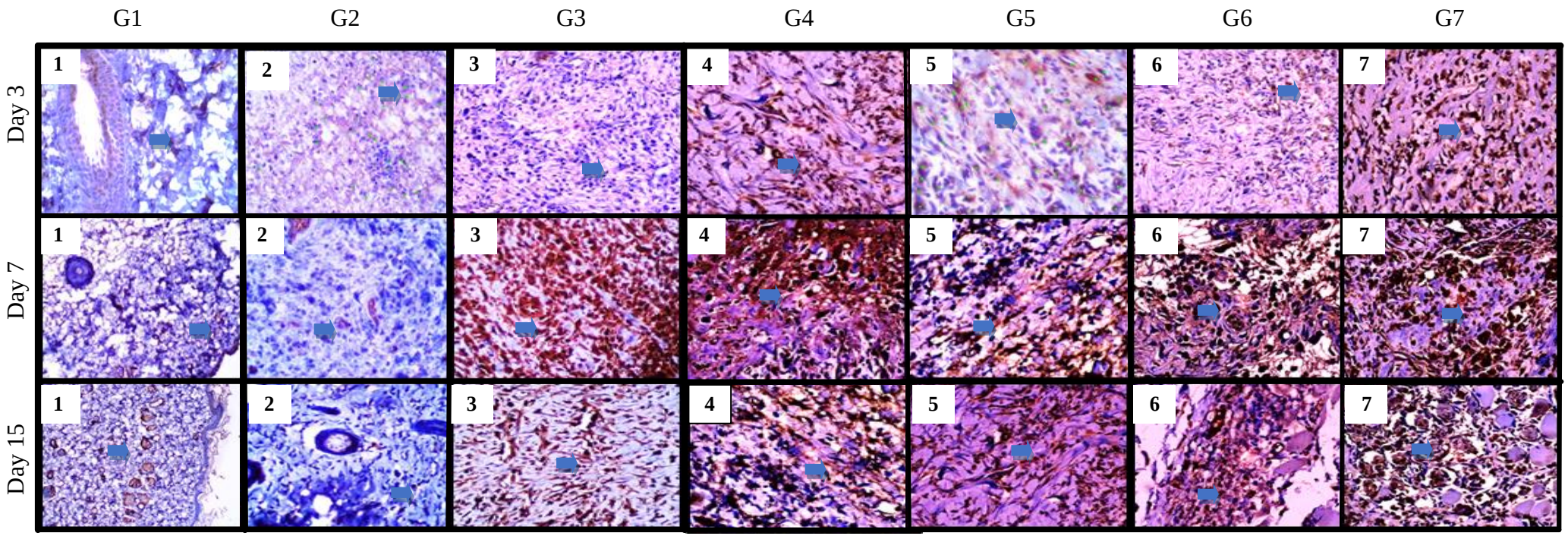

Figure 2. Histopathological description of fibroblast growth factor (FGF) expression using immunohistochemical staining of skin tissue on the backs of Sprague Dawley rats in various groups on days 3,7 and 15 with 400x magnification. **G1:** Negative control; **G2:** Distilled water; **G3:** Betadine 10 %; **G4:** Gel base; **G5:** Gel base + sodium metabisulfite 0.2 gr; **G6:** Gel base + *Aloe vera* 5%; and **G7:** Gel base + *Aloe vera* 5% + sodium metabisulfite 0.2 gr

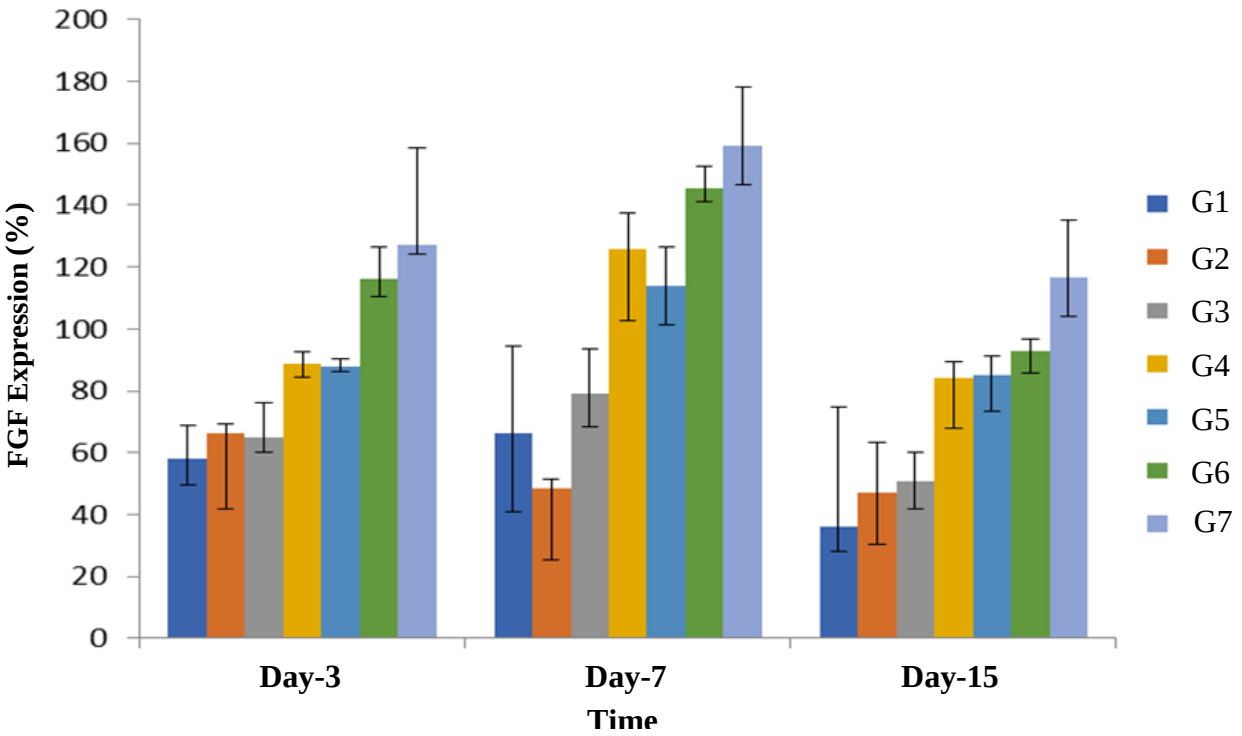

Figure 3. Comparison of fibroblast growth factor (FGF) expressions. **G1:** Negative control; **G2:** Distilled water; **G3:** Betadine 10 %; **G4:** Gel base; **G5:** Gel base + sodium metabisulfite 0.2 gr; **G6:** Gel base + *Aloe vera* 5%; and **G7:** Gel base + *Aloe vera* 5% + sodium metabisulfite 0.2 gr

The image shows a comparison of FGF expressions among different treatment groups on days 3, 7, and 15. Data are presented as median values, with minimum and maximum ranges. On day 3, G7 exhibited the highest FGF expression compared to other groups, while G1 had the lowest expression ($p < 0.05$). G2 did not differ significantly from G3 ($p > 0.05$), and there was no significant difference between G4 and G5 ($p > 0.05$). By day 7, the highest FGF expression remained in G7, followed by G6, while G5 had a lower FGF expression compared to G4 ($p < 0.05$). G3 showed a higher expression compared to G2 and G1. On day 15, FGF expression in G7 was still the highest, with G1 maintaining the lowest expression. G2 did not show a significant difference compared to G3 ($p > 0.05$). Also, there was no significant difference between G4 and G5. These results indicate that the addition of *Aloe* vera and sodium metabisulfite (AVM) significantly increases FGF expression compared to other treatments (Figure 3).

Fibroblast growth factor-2 (FGF-2) plays a crucial role in angiogenesis in wounds, the formation of granulation tissue, and the regulation of re-epithelialization (Takaya et al., 2022). Besides activating fibroblasts, FGF-2 activates other cells originating from the mesoderm, including vascular endothelium and smooth muscle cells, osteoblasts, and chondrocytes. FGF-2 can also accelerate the healing of acute and chronic wounds (Cowin, 2019). In this study, FGF expressions increased in all groups on days 3 and 7 but decreased on day 15. FGF-2 increases in the acute phase of wounds because it plays a role in the formation of granulation tissue, re-epithelialization, and tissue remodeling. FGF-2 also regulates the synthesis and deposition of extracellular matrix components, increasing keratinocyte movement, accelerating fibroblast migration, and stimulating collagen production. FGF-2 levels decreased in the chronic wound phase. This is characterized by an increase in FGF and fibroblasts on day 3 and reaching a peak on day 7 (Wei et al., 2022). Other studies have shown that local administration of FGF-2 can have anti-fibrotic effects in wounds, fighting myofibroblast differentiation and reducing fibrosis in wound tissue (Koike et al., 2020).

The results of this study are in line with several previous studies. In the study by Koike et al. (2020), local administration of FGF-2 also had anti-fibrotic effects showing the same functions in fighting myofibroblast differentiation and reducing fibrosis in wound tissue. They also reported that FGF-2 stimulates re-epitheliation in epidermal defect wound models. The results of the study by Takzaree et al. (2016) pointed to a significant increase in fibroblast formation and accelerated wound healing in the group that received *Aloe vera* gel, accompanied by an increase in TGF-β and FGF gene expression. Moreover, Atiba et al. (2022) found that topical application of *Aloe vera* gel not only accelerated wound healing but also increased the expression of vascular endothelial growth factor, basic fibroblast growth factor, and antioxidants. Additionally, Hormozi et al. (2017) reported that the administration of *Aloe vera* increased the expression of TGFβ1 and FGF *in vitro.*

To cite this paper: Purnamasari YW, Juniarti, Rahmah NA, Ernanda H, and Sari W (2024). Effect of *Aloe vera* Gel and Sodium Metabisulphite on Expression of Fibroblast Growth Factor in Incision Wound of Rats. *World Vet. J.,* 14(3): 311-317. DOI: https://dx.doi.org/10.54203/scil.2024.wvj37

CONCLUSION

The combination of *Aloe vera* L. extract gel at 5% concentration and sodium metabisulfite (0.2 gr) was more effective in increasing FGF expression than *Aloe vera* or sodium metabisulfite alone in the treatment of incision wounds on rat backs. Future research should consider comparing optimal treatment durations, examining different concentrations of *Aloe vera* extract, exploring the long-term effects of *Aloe vera* gel and sodium metabisulfite, and testing different wound models.

DECLARATIONS

Acknowledgments

All authors acknowledge the research participants at the Animal Research Facility, Yarsi University for their assistance.

Authors' contributions

Yulia Wiji Pirnama Sari performed an experiment and data analysis. Muhammad Hafid Ernanda designed and drafted the manuscript. Juniarti designed monitored, evaluated the data analysis, and revised the draft of the manuscript. Nunung Ainur Rahmah and Wening Sari performed data analysis and corrected the paper. The last edition of the manuscript was read and approved by all authors.

Funding

This study did not receive any financial support.

Competing interests

The authors have no conflict of interest.

Ethical considerations

This paper was originally written by the authors and has not been published elsewhere. The authors checked the text of the article for plagiarism index and confirmed that the text of the article is written based on their original scientific results.

Availability of data and materials

The data to support this study finding is available upon reasonable request to the corresponding author.

REFERENCES

Alturkistani HA, Tashkandi FM, and Mohammedsaleh ZM (2015). Histological stains: A literature review and case study. Global Journal of Health Science, 8(3): 72-79. DOI: https://www.doi.org/10.5539/gjhs.v8n3p72

Amiruddin, Syafruddin, Zuraidawati, Desky R, Siregar TN, Sayuti A, and Harris A (2015). The effect of papaya latex (*Carica papaya*) latex and povidone iodine on castration wound healing of male cat (*Felis domestica*). Jurnal Medika Veterinaria, 9(1): 44-47. DOI: https://www.doi.org.10.21157/j.med.vet..v9i1.2994

Atiba A, Abdo WS, kamal Ali E, Abd-Elsalam MM, Amer M, Monsef AA, Taha RS, Antar SA, and Mahmoud AI (2022). Topical and oral applications of *Aloe vera* improve healing of deep second-degree burns in rats via modulation of growth factors. Biomarkers, 27: 608-617. DOI: https://www.doi.org/10.1080/1354750x.2022.2085800

Baldi A, Sommella E, Campiglia P, and Daglia M (2021). *Aloe* gel-base food products: Chemical, toxicological, and regulatory aspects. Regulatory Toxicology and Pharmacology, 119: 104818. DOI: https://www.doi.org/10.1016/j.yrtph.2020.104818

Cowin AJ (2019). New innovations in wound healing and repair. International Journal of Molecular Sciences, 20(7): 1724. DOI: https://www.doi.org/10.3390/ijms20071724

Haid B, Pechriggl E, Nägele F, Dudas J, Webersinke G, Rammer M, Fritsch H, and Oswald J (2020). FGF8, FGF10 and FGF receptor 2 in foreskin of children with hypospadias: An analysis of immunohistochemical expression patterns and gene transcription. Journal of Pediatric Urology, 16(1): 41.e1-41.e10. DOI: http://www.doi.org.10.1016/j.jpurol.2019.10.007

Hekmatpou D, Mehrabi F, Rahzani K, and Aminiyan A (2019). The effect of *Aloe vera* clinical trials on prevention and healing of skin wound: A systematic review. Iranian Journal of Medical Sciences, 44(1): 1-9. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6330525/

Hormozi M, Assaei R, and Boroujeni MB (2017). The effect of *Aloe vera* on the expression of wound healing factors (TGFβ1 and bFGF) in mouse embryonic fibroblast cell: *In vitro* study. Biomedicine & Pharmacotherapy, 88: 610-616. DOI: https://www.doi.org/10.1016/j.biopha.2017.01.095

Ilie-Mihai RM, Ion BC, and van Staden JKF (2022). Sodium metabisulfite in food and biological samples: A rapid and ultra-sensitive electrochemical detection method. Micromachines, 13(10). DOI: https://www.doi.org/10.3390/mi13101707

Kartika AA, Siregar HC, and Fuah AM (2013). Business development strategy raising rats (*Rattus noevegicus*) and mice (mus musculus) at the faculty IPB animal husbandry. Journal of Production Science and Product Technology Farm, 1(3): 147-154. Available at: https://journal.ipb.ac.id/i ndex.php/ipthp/article/view/15543

Koike Y, Yozaki M, Utani A, and Murota H (2020). Fibroblast growth factor 2 accelerates the epithelial-mesenchymal transition in keratinocytes during wound healing process. Scientific Reports, 10(1): 1-13. DOI: https://www.doi.org/10.1038/s41598-020-75584-7

Kristantri R, Sari W, and Pebriani TH (2022). Test the total plate number (ALT) and yeast mold number (AKK) of sunscreen spray gel preparations for ethanol extract of cinnamon bark (Cinnamomum burmanii Ness. BI. Syn). Journal of Pharmaceutical Sciences, 3(2): 300-301. Available at: https://journal.ummat.ac.id/index.php/farmasi/article/view/9397/pdf

LaiCheong JE and McGrath JA (2017). Structure and function of skin, hair and nail. Journal Medicine Abingdon, 41(6): 317-320. DOI: https://www.doi.org/10.1016/j.mpmed.2013.04.017

Mustaqim A, Asri A, and Almurdi (2018). Effect of administering *Aloe vera* gel (*Aloe vera*) on the histopathological picture of the stomach of Wistar rats induced by indomethacin. Andalas Health Journal, 6(3): 641-645. DOI: https://www.doi.org/10.25077/jka.v6.i3.p641-646.2017

Nurhidayanti N (2022). The effectiviness test of *Aloe vera* extract inhibiting the growth of *Staphylococcus aureus* bacteria. Jurnal Biologi Lingkungan Industri Kesehatan, 8(2): 146-151. DOI: https://www.doi.org/10.31289/biolink.v8i2.5942

Retnowati D, Sari R, Hendradi E, and Septiani S (2021). The stability and irritability study of the chitosan-*Aloe vera* spray gel as wound healing. Journal of basic and clinical physiology and pharmacology, 32(4): 651-656. DOI: https://www.doi.org/10.1515/jbcpp-2020-0407

Sotoudeh N and Namavar MR (2022). Optimisation of ketamine-xylazine anaesthetic dose and its association with changes in the dendritic spine of CA1 hippocampus in the young and old male and female Wistar rats. Veterinary Medicine and Science, 8(6): 2545-2552. DOI: https://www.doi.org/10.1002/vms3.936

Takaya K, Aramaki-Hattori N, Sakai S, Okabe K, Asou T, and Kishi K (2022). Fibroblast growth factor 7 suppresses fibrosis and promotes epithelialization during wound healing in mouse fetuses. International Journal of Molecular Sciences, 23(13): 7087. DOI: https://www.doi.org/10.3390/ijms23137087

Takzaree N, Hadjiakhondi A, Hassanzadeh G, Rouini MR, Manayi A, and Zolbin MM (2016). Transforming growth factor-β (TGF-β) activation in cutaneous wounds after topical application of *Aloe vera* gel. Canadian Journal of Physiology and Pharmacology, 94(12): 1285-1290. DOI: https://www.doi.org/10.1139/cjpp-2015-0460

Wei L, Tan J, Li L, Wang H, Liu S, Chen J, Weng Y, and Liu T (2022). Chitosan/Alginate hydrogel dressing loaded FGF/VE-cadherin to accelerate full-thickness skin regeneration and more normal skin repairs. International Journal of Molecular Sciences, 23(3): 1249. DOI: https://www.doi.org/10.3390/ijms23031249

Wilantari P (2020). Incision wound healing activity from binahong leaf ointment (*Anredera scandens* (L.) moq.). Journal Udayana Pharmacy, 8(2): 78-89. DOI: https://www.doi.org/10.24843/jfu.2019.v08.i02.p04

Yusuf AL, Nugraha D, Wahlanto P, Indriastuti M, and Lestari NI (2020). Activity test of *Aloe Vera* skin extract gel for healing light burns in rabbits (*Oryctolagus cuniculus*). Jurnal Wiyata, 7(2): 133-137. Available at: https://ojs.iik.ac.id/index.php/wiyata/article/view/379