

Topical Modified-Olive Oil (Dalethyne) for Bacterial-Infected Wound: A Study in the Rats

Wawaimuli Arozal¹, Gregorius Bhaskara Wikanendra², Melva Louisa^{1*}, Kayapan Satya Dharshan³,
Kusmardi Kusmardi⁴, Ari Estuningtyas¹, Donna Savitry⁵

1. Department of Pharmacology & Therapeutics, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.
2. Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.
3. Dermozone Pratama, Jakarta, Indonesia.
4. Department of Anatomical Pathology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.
5. Plastic Surgery Subdivision, Bhayangkara, Sukanto Central Police Hospital, Jakarta, Indonesia.

Abstract

Open skin wound often leads to the infiltration of pathogens. Inadequate wound healing process might lead to systemic infections. Therefore, proper wound care is needed to prevent further possible complications. This study was aimed to investigate the effect of dalethyne administration on the wound healing process in open skin wounds infected by bacterias.

We conducted an in vivo experiment in rats. The skin of the rats was cut with a 1-cm incision along the torso on the left, and the right side then was inoculated with *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus epidermidis* and *Escherichia coli* for 24-hour. Afterward, vehicle (glycerine) or dalethyne were administered to the wounds. Macroscopic and microscopic observations were done on Day 1 and Day 6 after treatment administration.

Dalethyne application resulted in a significant wound closure rate compared with the vehicle only (glycerine) on skin wounds infected with *Pseudomonas aeruginosa*. No significant difference in wound closure rate for skin infected with *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *E.coli* after topical administration with dalethyne versus vehicle (glycerine). However, microscopic observation showed a higher tendency of the healing process, as demonstrated by neutrophil and mononuclear infiltration in the wound areas.

Dalethyne showed a beneficial healing rate in a rat model of bacteria-infected wounds. However, due to the short term observations in the present study, the more prolonged wound healing effect of dalethyne, still need to be investigated.

Experimental article (J Int Dent Med Res 2020; 13(3): 1223-1228)

Keywords: Modified olive oil, dalethyne, skin wound, antibacterial.

Received date: 23 June 2020

Accept date: 27 July 2020

Introduction

The skin wound is an injury on the skin caused by trauma, tear, cut, or incision or wound. When the skin is impaired, pathogens can infiltrate the skin so that it will be susceptible to infections.¹ The most common pathogens infecting the skin includes *Staphylococcus aureus*, *Streptococcus* sp., and *Pseudomonas aeruginosa*.^{2,3}

Many strategies have been applied to manage open wounds so that the skin repair will result in the restoration of tissue integrity and prevent further systemic infection.¹ Improper wound healing process might lead to severe damage, such as the loss of skin, inadequate blood flow to the epidermis, and aggravation of inflammatory state that will eventually lead to chronic wounds.⁴ Studies have shown that wound dressings containing topical antiseptics or antibiotics are an excellent option to limit the growth of bacterias and accelerate the process of wound healing. With growing resistance in pathogens against antimicrobial and antiseptic agents, the development of new topical antiseptic is urgently needed.^{4,5}

Studies have shown that olive oils and ozonated olive oils were shown to have a good effect on skin wound healing as well as

*Corresponding author:

Melva Louisa,
Department of Pharmacology and Therapeutics, Faculty of
Medicine, Universitas Indonesia.
Jl. Salemba Raya no 6, Jakarta Pusat, Jakarta Indonesia.
E-mail: melva.louisa@gmail.com

antimicrobial activity.⁶⁻⁹ Dalethyne is an ozonated olive oil, first introduced in India in 2015.^{10,11} Dalethyne has 18 side chains, categorized into four major groups, which are peroxides, aldehydes, iodines, and anisidines. Peroxides, iodines, and anisides are active compounds that helped in the process of wound cleaning in epidermis and dermis. This mechanism will help in creating a conducive environment for tissue repair. Peroxides also can induce macrophage and neutrophil activities and aides in the process of phagocytosis and debridement. Meanwhile, the aldehyde group of dalethyne worked in the hypodermic layer.¹⁰

The result from *the in silico* study demonstrated that dalethyne could inhibit the growth of various positive and negative gram bacteria. The findings from *in silico* study shown the promising potential of dalethyne as a broad-spectrum antibacterial agent.¹² Dalethyne also has other beneficial activities such as suppressing inflammation, oxidative stress, and modulating growth and inflammatory factors, which will help towards wound healing.¹⁰ Previous *in vivo* studies had also reported the acceleration of wound healing in MRSA-infected rats.¹³ Therefore, this study was aimed to investigate the acute effect of dalethyne administration on the wound healing process in open skin wounds infected by bacterias in the rats.

Materials and methods

Design

This study was an *in vivo* experiment conducted using Sprague-Dawley male rats, 3-months old weighing about 150 – 200 grams. The Ethics Committee has approved the study before the start of the research (No. 0041/UN2.F1/ETIK/2019). Incision wounds were inoculated with four types of bacterias, which were *Staphylococcus aureus* (SA), *Pseudomonas aeruginosa* (PA), *Staphylococcus epidermidis* (SE) and *Escherichia coli* (EC). The standard group consists of rats that were incised but not inoculated with bacterias. Three pairs of incisions were done in each rat, which was the vehicle (glycerine) in the left part of the incisions and dalethyne in the right part of the incisions. Every treatment consists of six samples.

The rats were kept at stable room temperature ($\pm 25^{\circ}\text{C}$), with 12 hours of light/dark

cycle. The rats received standard pellets for food and water ad libitum. During the treatment period, each rat was kept on an individual closed-system cage to prevent cross-infection.¹⁴

Incision Procedure

Rats were put under anesthesia by intramuscular injection of ketamine (80 mg/kg BW) and xylazine (8 mg/kg BW). Afterward, the rat fur was shaved in the incision area. Three pairs of 1-cm incisions were made along the torso on both the left and right side with 1.5 cm distance for each incision using a biopsy punch. The gauze was then put to cover the incision.¹⁵

Microbes Inoculation

Inoculation of the microbes was done after the incision. Before microbial application, the incision was irrigated using normal saline. Inoculation was done by topically applied 0.2 mL of a bacterial suspension of 4.12×10^9 CFU/mL on the site of the incision. Bacterias were obtained from the University of Pancasila. The normal group received phosphate buffer solution (PBS) instead of a microbe suspension. After inoculation, the incision was covered using sterile gauze.¹⁵

Treatment Application

Twenty-four hours after inoculation, the incision was then irrigated using normal saline. Treatment was done by topically applying a placebo to the left side incisions and 30% dalethyne to the right-side incisions. Afterward, the incision was covered again using sterile gauze. The process was repeated every 24 hours for five days (Day 2 to 6).

Sample Collection

At the end of the study (Day 7), the rat was sacrificed to collect tissue samples. Rats were sacrificed under anesthesia injection of ketamine + xylazine.¹⁴ Necropsies were done after confirmation of death to collect tissues around the incision site. Tissue samples were then put into a 10% formaldehyde buffer to be processed for histopathological evaluation using Hematoxylin-Eosin staining.

Macroscopic Evaluation

A macroscopic evaluation was done daily by inspecting and documenting incision after irrigation but before the application of placebo or dalethyne. A blinded pathologist did the review. The assessment was conducted by measuring the diameter of the wound. The infected incision was defined by the wound surface that was covered by pus, spreading of necrotic tissue to

the surrounding area of the incision, lowered surface level of a wound, and lowered wound healing rate compared to the normal group.¹⁵

Microscopic Evaluation

Microscopic evaluation was done by examining the histopathological sample. A blinded pathologist conducted the assessment. Evaluated characteristics include epithelialization, fibroplasia, neovascularization, and wound closure.¹⁵

Statistical Analysis

The normality test was conducted using a One-sample Kolmogorov Smirnov test with 95% significance, and data was considered normal if $p \leq 0.05$. The homogeneity test was then conducted with 95% significance, and data were deemed to be homogenous if $p \leq 0.05$. Statistical analysis for parametric data was done using ANOVA with Tukey posthoc. Non-parametric data were analyzed using the Kruskal-Wallis test, followed by Mann-Whitney U.

Results

Observation and calculation conducted in this study have produced the result in the form of macroscopic and microscopic evaluation. The macroscopic evaluation was obtained by inspecting wound closure, as shown in Figure 1. Calculation of the wound closure rate percentages was done based on the difference of wound size from day one to the last day of the study (Figure 2).

Dalethyne tends to increase the wound closure rate, though not statistically significant, for uninfected incision and infected incision caused by *Staphylococcus aureus*, *Staphylococcus epidermidis* and *E.coli*. Dalethyne was found to increase the wound closure rate significantly for the incisions infected by *Pseudomonas aeruginosa*. We found a worsening in wound closure in incisions infected with *E.coli*, while in dalethyne groups, we found a small improvement in wound closure rate (Figure 2).

The macroscopic wound closure results were confirmed by microscopic evaluation done by examining Hematoxylin-eosin (HE) stained histopathological samples. A summary analysis of each group is given in Table 1, while the histopathological assessment is presented in Figure 3. We did not find differences in the

degree of dermatitis in the normal group treated with vehicle versus dalethyne. No specific abnormality or minimal necrosis was observed in *Escherichia coli*- and *Staphylococcus epidermidis*-infected skin treated with vehicle and dalethyne.

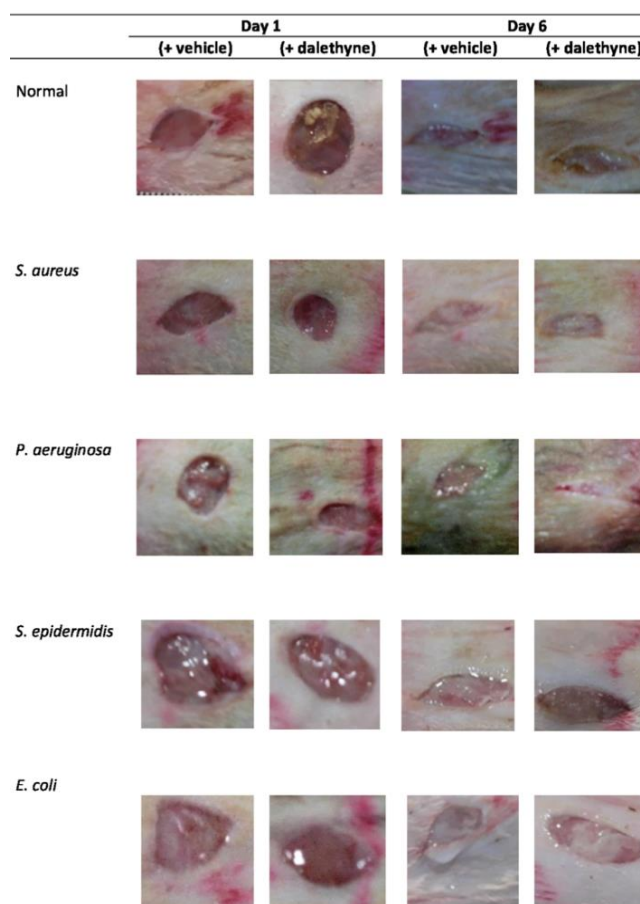


Figure 1. Macroscopic evaluation of wound closure. Photos were taken on Day 1 and Day 6.

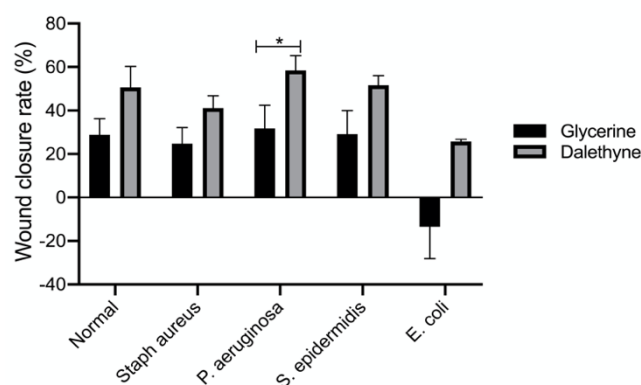


Figure 2. Wound closure rate of incision infected with bacteria when treated with vehicle only (Glycerine) or Dalethyne.

Results are given in mean \pm SEM. * : $p < 0.05$.

In wounds infected with *Staphylococcus aureus*, both groups, vehicles, and dalethyne showed dermal necrosis. In wounds infected with *Pseudomonas aeruginosa*, there were signs of cutaneous necrosis in the vehicle group, while in the dalethyne group, there were only signs of mild dermatitis.

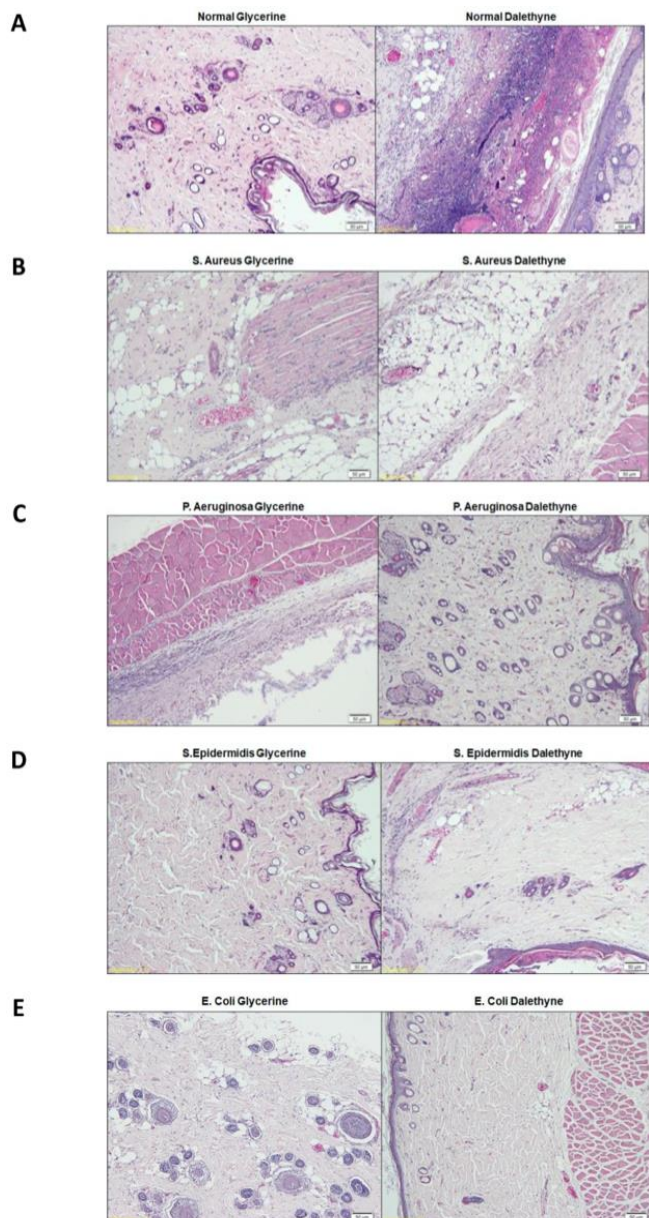


Figure 3. Histopathological evaluation at 100 x magnification of (A) control group; or bacteria-infected wounds (B) *Staphylococcus aureus*; (C) *Pseudomonas aeruginosa*; (D) *Staphylococcus epidermidis*; (E) *Escherichia coli*, after treatment with vehicle (glycerine) or dalethyne.

Discussion

The present study was aimed to evaluate the efficacy of dalethyne in the healing process of skin wounds infected with bacteria. Dalethyne is an ozonated olive oil with promising results in *in silico* study, and *methicillin-resistant staphylococcus aureus* (MRSA) wound infections.^{10,12,13} The ozonization of the olive oil might partly contribute higher wound closure rate in the dalethyne group. Ozonization of the olive oil was aimed to stabilize the double bonds of the monosaturated fatty acids in the olive oil. However, the ozone itself has been widely recognized to have bactericidal and fungicidal activity.^{5,16} Ozonides is known to penetrate the skin efficiently, therefore aiding wound healing and repair of the skin.^{5,17,18} Ozone has also been widely recognized as bactericidal and antifungal.^{7,8} It also has been suggested that the bond between O₃ and polyunsaturated fatty acids leads to the activation of transcription factors such as NF-κB that induce the synthesis of growth factors, therefore, increase the rate of wound healing.^{5,18}

Group	Results	Description
Normal glycerine	+ Minimal dermal necrosis	Epithelial necrosis on dermis, hemorrhage, congestion, hyalinization and mononuclear infiltration (lymphocyte).
Normal dalethyne	+ Minimal hemorrhagic dermatitis	Cutaneous epithelial necrosis, cellular debris, mineralization, neutrophil and mononuclear infiltration (lymphocyte and macrophage), hypercellular, fibrosis, hyalinization, congestion, hemorrhage, and hair follicle epithelialization.
<i>S. aureus</i> glycerine	+ Minimal dermal necrosis	Epidermal epithelial necrosis, hair follicle growth, skeletal muscle necrosis, mononuclear infiltration (lymphocyte and macrophage), the fat layer is thicker than epidermis and dermis, hyalinization, congestion and fibroblast.
<i>S. aureus</i> dalethyne	+ Dermal necrosis	Epidermis and dermis epithelial necrosis, muscle fiber fragmentation, and congestion.
<i>P. aeruginosa</i> + glycerine	Minimal dermal necrosis	Epidermis and dermis epithelial necrosis, skin tissue fragmentation, skin epithelium desquamation, mononuclear infiltration (lymphocyte and macrophage), hemorrhage and muscular hyalinization.
<i>P. aeruginosa</i> + dalethyne	Dermatitis	Endodermic necrosis, neutrophil and mononuclear infiltration (lymphocyte and macrophage), congestion and haemorrhage.
<i>S. epidermidis</i> + glycerine	NSA	No specific anomaly (NSA).
<i>S. epidermidis</i> + dalethyne	Minimal subcutaneous necrosis	No abnormality in epidermis and dermis, neutrophil and mononuclear infiltration (lymphocyte and macrophage) in skeletal muscle tissue and muscular fiber degeneration.
<i>E. coli</i> glycerine	+ NSA	No specific abnormality (NSA).
<i>E. coli</i> dalethyne	+ NSA	No specific abnormality (NSA).

Table 1. Microscopic Evaluation for All Treatment Groups.

Olive oils, known to be rich in unsaturated fatty acids, known to have potent antibacterial activities. Phenolic compounds in olive oils such as aliphatic aldehydes can delay the growth of a wide range of bacterias.^{7,12}

Out of the four types, bacterial skin infections studied, dalethyne gave the best activity against *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* is a drug-resistant strain of bacteria with a limited choice of antibiotics. Therefore, new treatment options to treat *Pseudomonas* spp. infection will be beneficial. In a previous study of *Pseudomonas aeruginosa* skin-infected rats, dalethyne was shown to reduce the inflammatory mediator IL-1 β in fibroblast cells on day four after treatments, which reduces the inflammatory phase of the wound healing process.¹¹

In treatment groups inoculated with *E. coli*, we observed worsening in placebo groups while in dalethyne groups, a small improvement was found. *E. coli* is a gram-negative bacteria that can cause debilitating skin and soft tissue infections in humans. *E-coli* produces non-specific exotoxin that disrupts intracellular signaling leads to cell deaths.¹⁹ The bacteria might have spread to adjacent areas, and cause the wound to widen. Dalethyne was shown to prevent the worsening of wound infection caused by *E. coli* and the spreading of the bacterias.

Overall, our microscopic findings confirmed what we found on the visible observation. No apparent differences microscopically in groups given vehicle (glycerine) or dalethyne in all groups. In the vehicle group, we found dermal necrosis, while in the dalethyne group, we found dermatitis, which is marked with neutrophil and mononuclear infiltration. In the wound healing process, neutrophils will be involved in the formation of the extracellular matrix. This process can be accelerated by administering wound care products.²⁰⁻²²

It was known that immediately after injury, local immune cells are activated, then release to the sites of the wound, proinflammatory mediators are released, neutrophils as the dominant white cells infiltrate quickly to the location of the injury.²³ Whereas mononuclear cells, along with cytokines, chemokines, and growth factors, actively participate in providing healing process.^{20,24} Circulating lymphocytes migrate to the wound area during the first 24

hours and will decline after a week. The release of neutrophil and mononuclear cells marks the acute phase of wound healing, while in the later stage, which includes tissue remodeling and formation of scar or fibrotic tissues, which can last weeks or months.^{14,20,23-24}

In the dalethyne group, the majority of the microscopic findings include neutrophil and mononuclear infiltration, which showed that the healing process was still in its early phase. In the placebo group, we observed more dermal necrosis along with congestion and hemorrhage.

However, we also observed epidermis and dermis epithelial necrosis in dalethyne groups. In our experiment, our observation only lasts for seven days, which are still early for complete wound healing. Therefore, for a comprehensive wound healing representation, it is best to have more extended observation to demonstrate the whole reepithelization process.

Conclusions

Taken together, we conclude the activity of dalethyne as an antibacterial in open wound skin infections. Dalethyne provides faster wound healing as compared to vehicle, in particular against *Pseudomonas aeruginosa* infected wounds. Dalethyne tends to show a higher wound healing rate compared to the vehicle against *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *E. coli*. However, due to the short period of observations in the present study, more prolonged effects of dalethyne is still needed to be investigated.

Acknowledgements

The study was funded by the Grant from Directorate of Research and Community Engagement, Universitas Indonesia.

Declaration of Interest

The authors declare no conflict of interest.

References

1. Pazyar N, Yaghoobi R, Rafiee E, Mehrabian A, Feily A, Skin wound healing, and phytomedicine: A review. *Skin Pharmacol Physiol*, 2014; 27: 303–10.
2. Aly R. Microbial Infections of Skin and Nails. In: Baron S, ed. *Medical Microbiology*. 4th ed, 1996. University of Texas Medical Branch at Galveston. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK8301/>

3. Stulberg DL, Penrod MA, Blatny RA, Common Bacterial Skin Infections. *Am Fam Physician*. 2002; 66: 119.
4. Negut I, Grumezescu V, Grumezescu AM. Treatment Strategies for Infected Wounds. *Molecules*, 2018; 23(9): 2392.
5. Kim HS, Noh SU, Han YW, Kim KM, Kang H, Kim HO, et al. Therapeutic Effects of Topical Application of Ozone on Acute Cutaneous Wound Healing. *J Korean Med Sci*. 2009; 24: 368–74.
6. Carata E, Tenuzzo BA, Dini L. Powerful Properties of Ozonated Extra Virgin Olive Oil. In: Builders PF, ed. *Herbal Medicine*. IntechOpen, 2018. Available from: <https://doi.org/10.5772/intechopen.73211>.
7. Karaosmanoglu H, Soyer F, Ozen B, Tokatli F. Antimicrobial and antioxidant activities of Turkish extra virgin olive oils. *J Agric Food Chem*. 2010; 58: 8238–45.
8. Medina E, Romero C, Brenes M, De Castro A. Antimicrobial activity of olive oil, vinegar, and various beverages against foodborne pathogens. *J Food Prot*. 2007; 70: 1194–9.
9. Uysal B. Ozonated olive oils and the troubles. *J Intercult Ethnopharmacol*. 2014; 3: 49–50.
10. Dharshan K. Overview of Dalethyne and other topical antiseptics for wound care. *Greener J Biomed Health Sci*. 2018; 4: 11–8.
11. Dharshan K. Effect of +Dalethyne on *Pseudomonas aeruginosa*. *Int J Pharm Pharm Res*. 2018;12: 258–66.
12. Dharshan K. Docking Studies for Assessment of Wound Healing Potential of Dalethyne Derivatives: An in silico Approach. *Computational Biol Bioinformatics*. 2018; 6(2): 36 – 51.
13. Dharshan K. +D Alethyne potential toward epithelialization of wound on the skin of MRSA infected rats. *J Chem Pharm Res*. 2018; 10: 187–97.
14. Dorsett-Martin WA. Rat models of skin wound healing: a review. *Wound Repair Regen*, 2004; 12: 591–9.
15. Asada M, Nakagami G, Minematsu T, Nagase T, Akase T, Huang L, et al. Novel models for bacterial colonization and infection of full-thickness wounds in rats. *Wound Repair Regen*. 2012; 20: 601–10.
16. Güneş S, Bahsi E, İnce B, Çolak H, Dalli M, Yavuz İ, et al. Comparative Evaluation of the Effects of Ozone, Diode Laser, and Traditional Cavity Disinfectants. *Microleakage, Ozone-Sci Eng*. 2014; 36: 206–11.
17. Gajendrareddy PK, Sen CK, Horan MP, Marucha PT. Hyperbaric oxygen therapy ameliorates stress-impaired dermal wound healing. *Brain Behav Immun*. 2005; 19: 217–22.
18. Valacchi G, Lim Y, Belmonte G, Miracco C, Zanardi I, Bocchi V, et al. Ozonated sesame oil enhances cutaneous wound healing in SKH1 mice. *Wound Repair Regen*. 2011; 19: 107–15.
19. Ki V, Rotstein C. Bacterial skin and soft tissue infections in adults: A review of their epidemiology, pathogenesis, diagnosis, treatment, and site of care. *Can J Infect Dis Med Microbiol*. 2008; 19: 173–84.
20. Solikhah I, Widiyanti P, Aminatun. Composition of chitosan-gelatin scaffolds with glutaraldehyde cross linker for skin tissue engineering in burn wound cases. *J Int Dent Med Res*. 2018; 11(13): 778 – 85.
21. Budi SH, Astuti ER. The MMP-2, MMP-9 expression, and collagen density of the Ambonese banana stem sap administration on wound healing. *J Int Dent Med Res*. 2019; 12(2): 492 – 7.
22. Hanafiah OA, Abidin T, Ilyas S, Nainggolan M, Syamsudin E. Wound healing activity of binahong (*Anredera cordifolia* (Ten.) Steenis) leaves extract towards NIH-3T3 fibroblast cells. *J Int Dent Med Res*. 2019; 12(3): 854 – 8.
23. Koh TJ, DiPietro LA. Inflammation and wound healing: the role of the macrophage. *Expert Rev Mol Med*. 2011; 13: e23.
24. Cañedo-Dorantes L, Cañedo-Ayala M, Skin Acute Wound Healing: A Comprehensive Review. *Int J Inflamm*. 2019; 2019:3706315.