

The Episiotomy Effect of Topical Combination of Cinnamon Oil and Red Betel on Skin Wound Healing Mechanism

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ABSTRACT

The process of episiotomy surgery during childbirth results in injury and pain in the perineum. This study aims to evaluate the effect of the topical combination of cinnamon oil and red betel extract on the healing process of episiotomy incision model in Swiss mice. The research subjects were divided into six groups: (I) Negative control, (II) positive control, (III) mice treated with cinnamon oil, (IV) mice treated with red betel extract, (V) mice treated with 2 ml cinnamon oil and 20% red betel extract, and (VI) mice treated with 4 ml cinnamon oil and 40% red betel extract. Each group of mice received a full-thickness skin wound on the back. The incision wound was then applied by using gel two times a day for 5 days which had been prepared previously. The results showed that the combination of cinnamon oil and red betel decreased the area and percentage of the wound, decreased the inflammatory cell infiltration, and increased the vascular endothelial growth factor expression. This study concludes that the combination of cinnamon oil and red betel extract have the potential to accelerate wound healing and reduce perineal pain in episiotomy incision models conducted in Swiss mice.

Keywords: Cinnamon oil, Red betel, Episiotomy, Skin wound healing, Perineum pain

1. INTRODUCTION

Episiotomy is a surgical process by making an incision in the vaginal opening and perineum to facilitate the childbirth process [1][2]. A tear due to an episiotomy is equivalent to a second-degree perineal tear in spontaneous labour, that is the injury to the perineum, especially to the perineal skin, vaginal mucosa, and perineal muscles [3]. Perineal trauma is common in Asian countries including Indonesia because the condition of the perineal tissue in Asian women is relatively short [4]. The process of episiotomy surgery results in injury and pain of the perineum. Pain due to episiotomy is higher than that of non-episiotomy [5]. More than 90% of perineal pain can occur on the first day of childbirth, and as many as 88% of pain cases will subside within 2 months after childbirth. Pain in the perineum will affect the life quality of the mother, and can interfere the relationship between mother and child, which then interfere the successful breastfeeding [6][7].

Poor handling of perineal wounds can also result in infection of the wound which is characterized by an increase in temperature, pain, heat, redness, ecchymosis, as well as perineal abscess and can lead to a slow healing process [8][9]. There are several ways that can be carried out to speed up the healing process and to relieve pain in the perineal wound such as acupuncture, the use of green tea ointment, the use of non-steroidal anti-inflammatory

drugs (NSAID), and even the use of antibiotics if the wound reaches the stage of infection [10][11]. Wound healing can through several mechanisms, such as reducing inflammation reactions in wounds, supporting tissue re-epithelialization, reducing oxidative stress, increasing oxygen levels, supporting angiogenesis, increasing fibroblast migration up to the mechanism of eliminating the presence of microorganisms in the wound [12].

Although the use of NSAID is effective in reducing pain in the perineum, it also interferes with the wound healing process due to the side effects it causes. Povidone-iodine is also often used as an antiseptic in cases of perineal wounds [13]. However, it does not significantly reduce microorganisms, and the odour is not favourable [14]. The use of herbs is one of the methods of choice for this treatment. Herbal medicine such as cinnamon can be used in recovery treatment after an episiotomy. It can reduce perineal pain and improve the healing of episiotomy incisions [15]. The main components contained in cinnamon are cinnamaldehyde, eugenol, cinnzeylanin, cinzeylanol, arabinosilane, 2-hydroxynamalehide, and 2-benzoxinamaldehyde [16][17].

Red betel (*Piper betel* Var. *Rubrum*) contained with essential oil has antiseptic activity. A Red betel leaf infusion can heal perineal wounds with less total treatment days compared to betadine [18][19]. It is

possible that the combination of these two herbal ingredients can be used more effectively to accelerate the healing of episiotomy wounds and to reduce the occurrence of pain. However, research related to the combination of these two ingredients is not yet available; either it's pre-clinical or clinical research. Therefore, research regarding the combination of cinnamon oil and red betel extract to heal incisions performed in Vivo as the perineal wound model needs to be conducted.

2. METHODS

This research is a laboratory experimental study with a completely randomized design. The test was carried out in vivo using Swiss mice as test animals. The test begins with the extraction of red betel leaf using the maceration method. Meanwhile, cinnamon oil is obtained from Rumah Atsiri Indonesia. The material is then prepared into a topical gel preparation. Furthermore, experimental tests on mice were tested to observe the effect of topical preparations on wound width and continued with histopathological and immunohistochemical tests to observe the effect of administration of topical preparations on the number of inflammatory cells, VEGF antibody expression.

2.1. Red Betel Extraction

30 grams of red betel leaf extract was extracted by maceration by using 300 mL ethanol at room temperature (1:10) [20][21]. The maceration was carried out for two days with daily solvent replacement. The filtrate obtained was further filtered and evaporated with a rotary evaporator (40°C, 60rpm). The evaporated red betel leaf extract was stored at -20°C.

2.2. Animal Model and Experimental Design

A total of 30 female Swiss mice (2-3 months old, weighing 20-30 g) were used. The research subjects were divided into six groups and the following treatments were applied: group I (negative control), group II (positive control), group III (treated with gel containing Cinnamon oil), group IV (treated with gel containing red betel leaf extract), group V (treated with gel containing cinnamon oil 2 ml and red betel leaf extract 20%) and group VI (treated with gel containing 4 ml cinnamon oil and 40% red betel leaf extract). Each group of mice received full-thickness skin wound treatment on the back with surgical scissors after local anesthesia was given. The incision wound was then applied with a gel that had been prepared by giving it twice a day for 5 days and the width of the wound was measured from the 1st day until the 5th day. This study had obtained ethical clearance from Alma Ata University with registration number KE/AA/VI/10150/EC/2020.

2.3. Histopathology and Immunohistochemistry

Skin samples of the experimental animals were collected and stored in 10% neutral buffered formalin. The skin was then processed for histopathology. They were dehydrated and were cleared using alcohol and xylene and then were embedded in paraffin block. The blocks were cut using a microtome at 0,5 mm thickness. Furthermore, the tissue was stained using hematoxylin and eosin which aims to express tissue components and inflammatory cell infiltration. Immunohistochemical analysis of tissue specimens was carried out by using antibodies Vascular Endothelial Growth Factor (VEGF) (Santa Cruz Biotechnology, Inc VEGF(C-1): sc-7269).

2.4. Statistical Analysis

The data of episiotomy wound healing were presented in the form of a percentage (%) and the incision wound width were presented with centimeters (cm) by using mean \pm standard deviation (SD). Statistically, the clinical significance was determined by the statistical significance value of $p < 0.050$. A two-way ANOVA followed by the Tukey's post hoc analysis was used to determine the average difference in the percentage of the incision wound healing between groups divided into two variables based on the day of observation and the application of treatment. In addition, it also determines the significant interaction after treatment application with the day of observation on the width of the incision wound. Statistical analysis using one-way ANOVA analysis of variance and post hoc LSD to determine the differences in the mean values of neovascularization "new blood vessels" and VEGF expression between treatment groups. This is because of data collection the formation of new blood vessels and the expression of VEGF only on the 5th day.

3. RESULTS AND DISCUSSION

This study was conducted to examine the effect of a topical combination of cinnamon oil and red betel ethanol extract on mice receiving episiotomy incision model treatment. The results show that the mean width of the incision (Figure 1) experiences a gradual decline, especially in group VI and V. Based on the percentage of wound healing (Figure 2), the percentage of wound closure is statistically significant on day 3 to 5 ($p < 0.050$). The combination of cinnamon and red betel with different formula concentrations, where each ingredient in group 6 had a two-fold higher concentration, showed significantly different healing on day 5 with a better percentage of clinically healing wounds. This combination contained high antioxidant potential for managing oxidative stress caused by episiotomy wounds. Thus, it can accelerate the healing process of inflammation [22].

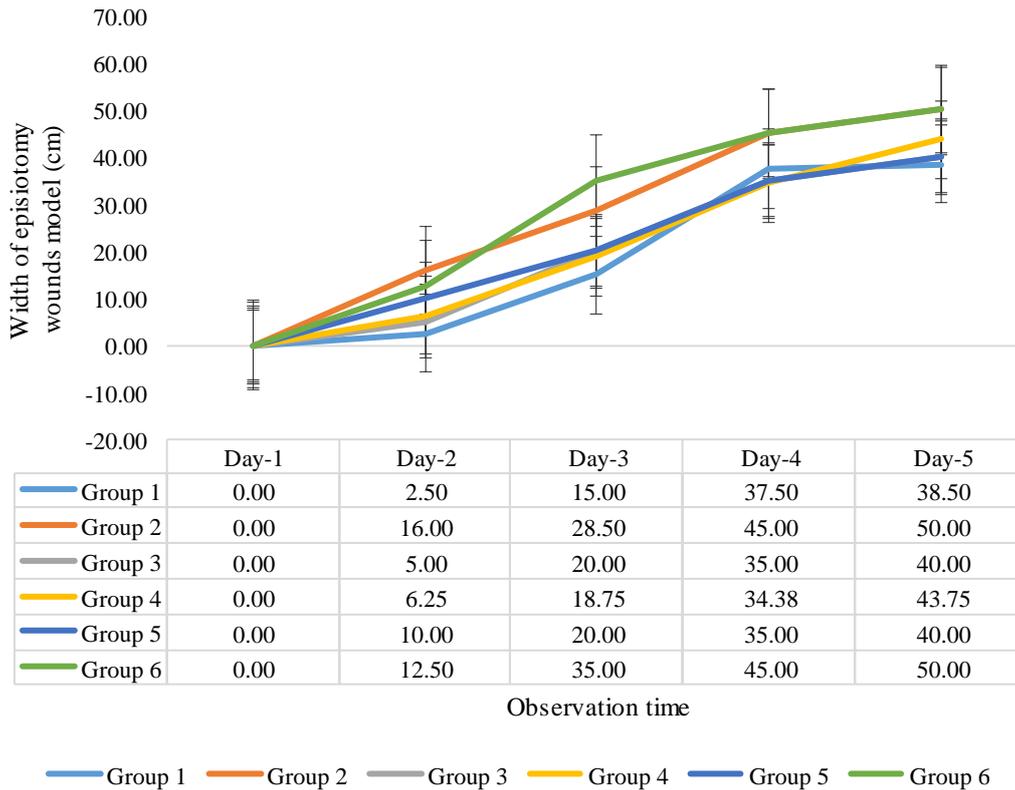


Figure 1. The effect of width of episiotomy wound model on treatment and observation time on day 1-5th

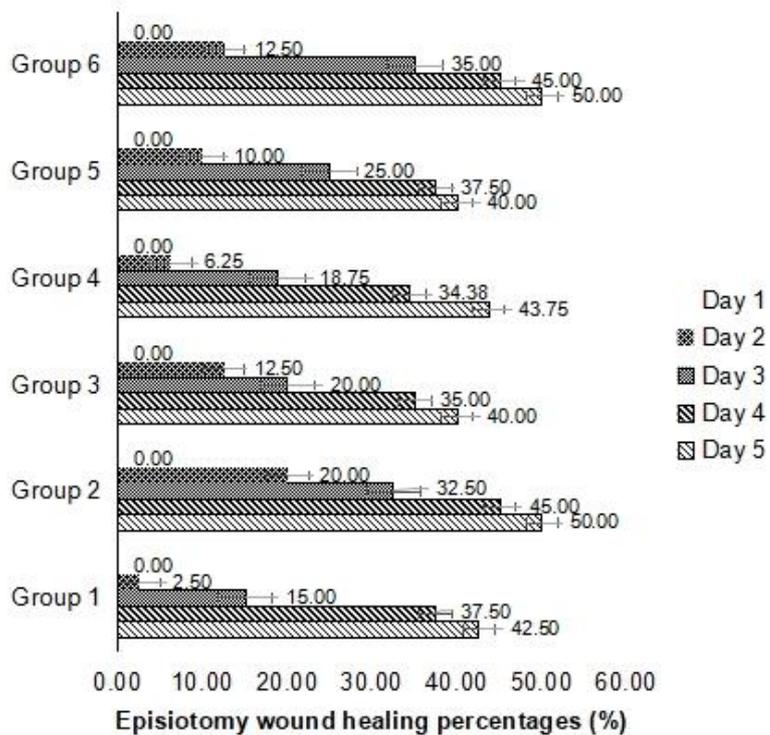


Figure 2. Means of episiotomy wound healing percentages between the study groups at different time points. Data are presented as the mean ± SE, with statistically significant ($p < 0.050$) differences between groups in the different day

Based on the width of the wound and the percentage of healing, the potency of giving a combination of

cinnamon oil and red betel is equivalent to povidone-iodine to assist wound healing and to inhibit bacterial

growth in the incision area with minimal side effects, especially in episiotomy cases. The percentage of healing results can also be ascertained from the width of the incision which gives a significant reduction, especially in the group 6. The healing percentage can also be ascertained from the width of the incision which gives a significant reduction, especially in the 6th group (Figure 2).

The results of the two-way analysis of variance show that each treatment, observation time and the interaction of the two have a significant impact on the width of the incision wound healing ($p < 0.001$). Combination of the application of cinnamon oil and red betel extract leaf gel with significant effect compared to the negative control group and monotherapy of red betel leaf extract and cinnamon oil on the width of incision in episiotomy wound healing modelling of Swiss mice. Interaction between application of treatment and the day of observation $F [79.634]$, $p < 0.001$; the day of observation $F [20.685]$, $p < 0.001$; application of treatment $F [55.920]$, $p < 0.001$; Figure 1). During the 5-day observation, the wound healing simulated by episiotomy was significantly improved every day ($p < 0.001$).

Based on the visual observations on the inflammation symptoms occurred due to impaired blood flow caused by tissue damage to the blood vessels, it

showed that group 2 and 6 had better healing process than that of the other groups. This was also supported by observations of the number of inflammatory cells which showed that there were significant differences between group 6 and other groups. The mean number of inflammatory cells in group 6 is significantly different from that of group 1, 2, 3, 4, and 5, with a significance value of $p < 0.001$ for each group (Figure 3). Meanwhile, the mean number of inflammatory cells in group 3, 4, and 5 shows no significant difference with that of group 1 ($p > 0.050$). Based on these statistical tests, it is known that group 6 has the lowest mean number of inflammatory cells compared to the whole group.

The low number of inflammatory cells on day 5 clinically indicated that the combination of 4 ml cinnamon oil and 40% of red betel leaf extract had anti-inflammatory activity by inhibiting the production of inflammatory mediators. The inhibitory effect of inflammation causes a decrease in vasodilation of blood vessels, so that the number of inflammatory cells that migrate to the inflammatory tissue will also decrease [23]. Its ability to reduce the number of inflammatory cells and wound healing was seen microscopically better than the administration of povidone-iodine.

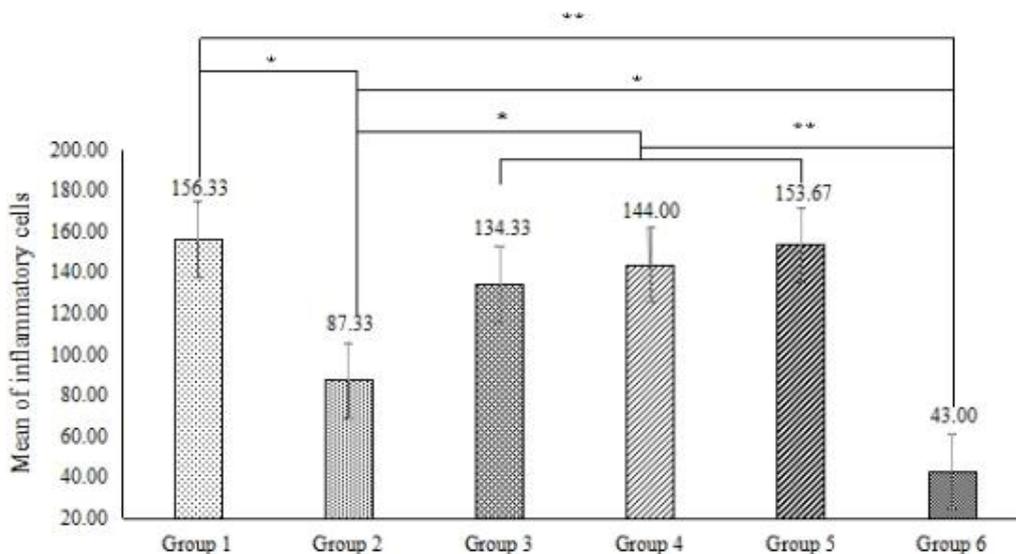
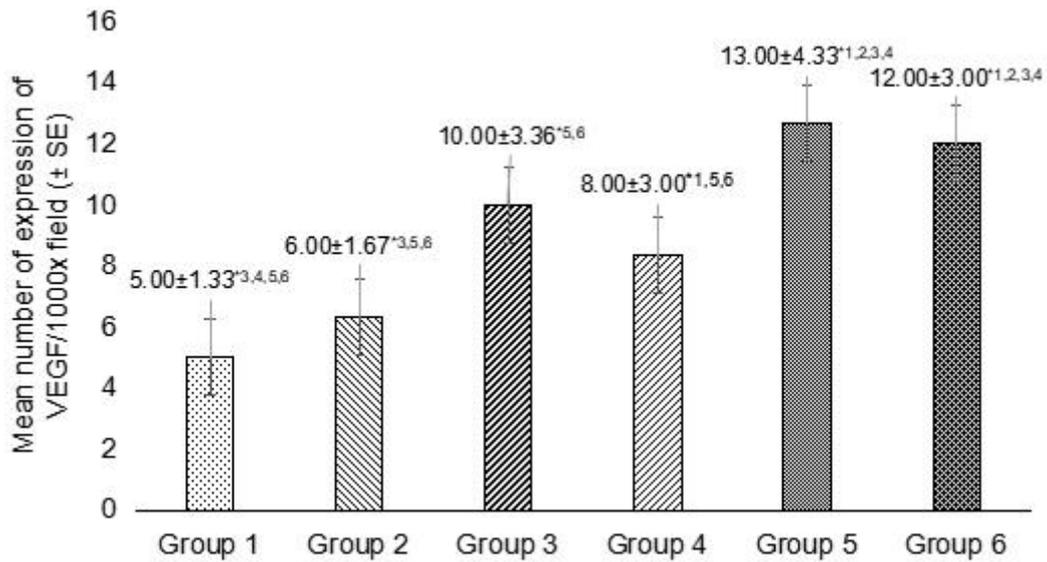


Figure 3. Inflammatory cells infiltration of episiotomy wound healing model on day 5 between the study groups; * $p < 0.05$ and ** $p < 0.001$, statistically significant difference with one-way ANOVA with LSD post hoc test

Perineal wound care with povidone-iodine antiseptic solution prevents post-partum infection and endometritis, and accelerates episiotomy wound closure after childbirth [24][25]. Administration of povidone-iodine at a concentration of 1/20 causes damage to fibroblasts and keratinocytes and inhibits lymphocytes, thereby delays the wound healing and increase the pain intensity [26][27]. In addition, the administration of povidone-

iodine is irritating and more toxic if it enters the blood vessels, especially when given with a concentration of more than 10% [28][29]. Therefore, the results of this study indicates that topical administration of 4 ml cinnamon oil and 40% of red betel leaf extract can be used as an alternative treatment for episiotomy wound care.



*significant difference in the mean amount of VEGF expression between study groups using One-way-Anova analysis ($p < 0.05$; 95% CI)

Figure 4. Expression of VEGF the study groups at different on day 5. Data are presented as the mean ± SE, statistically significant based on one-way ANOVA with LSD post hoc tests ($p < 0.050$) differences between groups

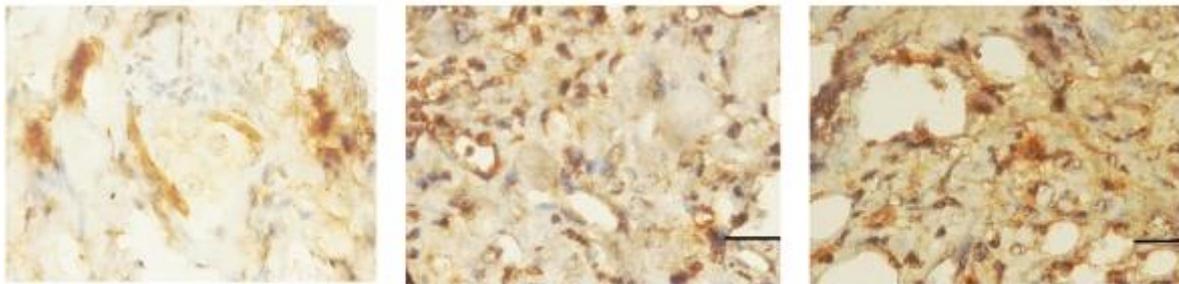


Figure 5. Expression of VEGF of episiotomy skin wound model on day 5 after treatment. There is no expression of the VEGF on the group 1 (a); the VEGF was expression in group 5 (b) and 6 (c). The positive reaction was indicated by the brown color of DAB. IHC antibody anti VEGF, 1000x

The decreasing signs of inflammation are also accompanied by an increase in the level of VEGF expression. The results shows that the response to wound healing is optimal in group 4, 5, and 6 ($p > 0.050$), and is significant in group 1 and 3 ($p < 0.050$). The formation of new blood vessels will affect the flow of blood vessels to the skin and mediates the migration/infiltration of cells, supply of nutrients, and oxygenation, so that the healing process will be faster. High vascularity is indicated by the presence of VEGF in scar tissue, which is one of the pro-angiogenic factors in wound healing that facilitates cell migration to the area of injury [30][31]. High VEGF expression causes repair in injured tissue. The expression level of VEGF is shown in Figure 4 and 5.

The potential of cinnamon for wound healing is attributed to its anti-inflammatory, analgesic, and antioxidant activities. Cinnamaldehyde is the main component found in cinnamon or about 45-65% is found in cinnamon essential oil [32] [33]. It is possible that the use of cinnamon oil can be more effective in healing wounds and perineal pain compared to the extract. In human skin disease models, cinnamon oil has anti-inflammatory activity by modulating gene expression, altering signaling pathways important in inflammatory processes, and tissue remodeling [34][17]. In addition, cinnamon also has antibacterial properties which decreases the risk of infection and manages perineal pain postpartum [35][36].

The effectiveness of cinnamon oil in healing wound and in reducing perineal pain can be optimized by combining cinnamon oil and red betel leaf extract. Red betel leaf extract can accelerate wound healing with its antiseptic and antibacterial properties. Red betel leaf contains as many as 23 components of chemical compounds, including 15 phenolic components, two monoterpenes, three sesquiterpenes, phenolic amide glycosides, neolignanes, and flavonoid c-glycosides [37][38]. The essential oils in red betel leaf include hydroxycyclicol, chavicol, cavibetol, allylprokatekol, carvacrol, eugenol, p-cymene, cineole, cariofelen, estragole cadimen, terpenes, and phenyl propada [19][39]. The content of alkaloids, saponins, tannins, and flavonoids contained in this plant is possible to stimulate the process of angiogenesis and the formation of epithelial cells [40][41]. The eugenol and cinnamaldehyde components contained in cinnamon oil will synergize with red betel extract which has the ability as an antiseptic so as to accelerate wound healing by accelerating the re-epithelialization process [15][42]. The secondary metabolites found in essential oils have biological activity in their mechanism of action on wounds by reducing retraction time and aiding healing.

4. CONCLUSION

The combination gel formulation of cinnamon oil and red betel extract can promote healing in mice treated with episiotomy wound models through its ability to reduce the number of inflammatory cell infiltration and to increase the expression of VEGF in the wound area.

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REFERENCES

- [1] K. Hartmann, M. Viswanathan, R. Palmieri, G. Gartlehner, J. Thorp, and K. N. Lohr, "Outcomes of routine episiotomy: A systematic review," *J. Am. Med. Assoc.*, vol. 293, no. 17, pp. 2141–2148, 2005, doi: 10.1001/jama.293.17.2141.
- [2] C. Schantz, K. L. Sim, E. M. Ly, H. Barennes, S. Sudaroth, and S. Goyet, "Reasons for routine episiotomy: A mixed-methods study in a large maternity hospital in Phnom Penh, Cambodia," *Reprod. Health Matters*, vol. 23, no. 45, pp. 68–77, 2015, doi: 10.1016/j.rhm.2015.06.012.
- [3] A. C. Act and A. C. Act, "Perineal injuries during childbirth," *J. Am. Coll. Cardiol.*, no. June, 2020.
- [4] H. Dahlen and C. Homer, "Perineal trauma and postpartum perineal morbidity in Asian and non-Asian primiparous women giving birth in Australia," *JOGNN - J. Obstet. Gynecol. Neonatal Nurs.*, vol. 37, no. 4, pp. 455–463, 2008, doi: 10.1111/j.1552-6909.2008.00259.x.
- [5] S. H. Soleimanzadeh Mousavi, M. Miri, and F. Farzaneh, "Episiotomy and Its Complications," *Zahedan J. Res. Med. Sci.*, vol. 23, no. 2, 2021, doi: 10.5812/zjrms.104127.
- [6] B. Torkan, S. Parsay, M. Lamyian, and A. Kazemnejad, "Postnatal quality of life in women after normal vaginal delivery and caesarean section," *BMC Pregnancy Childbirth*, vol. 9, pp. 1–7, 2009, doi: 10.1186/1471-2393-9-4.
- [7] J. Martínez-Galiano, A. Hernández-Martínez, J. Rodríguez-Almagro, and M. Delgado-Rodríguez, "Quality of Life of Women after Giving Birth: Associated Factors Related with the Birth Process," *J. Clin. Med.*, vol. 8, no. 3, p. 324, 2019, doi: 10.3390/jcm8030324.
- [8] R. K. Sharma and A. Parashar, "The management of perineal wounds," *Indian J. Plast. Surg.*, vol. 45, no. 2, pp. 352–363, 2012, doi: 10.4103/0970-0358.101318.
- [9] C. C. Chang *et al.*, "Risk factors for delayed perineal wound healing and its impact on prolonged hospital stay after abdominoperineal resection," *World J. Surg. Oncol.*, vol. 17, no. 1, pp. 1–7, 2019, doi: 10.1186/s12957-019-1768-4.
- [10] J. Hasegawa and L. C. Leventhal, "Pharmacological and non pharmacological treatment for relief of perineal pain after vaginal delivery Tratamento farmacológico e não farmacológico no alívio da dor perineal pós-parto normal," *Einstein*, vol. 7, no. 2 Pt 1, pp. 194–200, 2009.
- [11] H. Shahrahmani *et al.*, "The effect of green tea ointment on episiotomy pain and wound healing in primiparous women: A randomized, double-blind, placebo-controlled clinical trial," *Phyther. Res.*, vol. 32, no. 3, pp. 522–530, Mar. 2018, doi: 10.1002/ptr.5999.
- [12] J. S. Chin, L. Madden, S. Y. Chew, and D. L. Becker, "Drug therapies and delivery mechanisms to treat perturbed skin wound healing," *Adv. Drug Deliv. Rev.*, vol. 149–150, pp. 2–18, Sep. 2019, doi: 10.1016/J.ADDR.2019.03.006.
- [13] R. Gomaa, R. Farrag, S. Abd EL Raof Hashem, and R. Mohamed, "Effectiveness of Betadine versus Normal Saline Dressing on Episiotomy Wound Healing," *Egypt. J. Heal. Care*, vol. 10, no. 3, pp. 193–206, 2019, doi: 10.21608/ejhc.2019.58678.
- [14] World Health Organization, "WHO recommendations for prevention and treatment of maternal peripartum infections," *World Heal. Organ.*, p. 80, 2015.
- [15] A. Mohammadi, S. Mohammad-Alizadeh-Charandabi, M. Mirghafourvand, Y. Javadzadeh, Z. Fardiazar, and F. Effati-Daryani, "Effects of cinnamon on perineal pain and healing of episiotomy: A randomized placebo-controlled trial," *J. Integr. Med.*, vol. 12, no. 4, pp. 359–366, 2010.

- Jul. 2014, doi: 10.1016/S2095-4964(14)60025-X.
- [16] E. T. Wulandari and D. Kumalasari, "Herbal untuk Perawatan Masa Nifas: Penggunaan Kayu Manis untuk Nyeri Perineum dan Luka Episiotomi," *Aisyah J. Ilmu Kesehatan*, vol. (2), no. 2, pp. 93–98, 2017.
- [17] N. Błaszczuk, A. Rosiak, and J. Kałużna-Czaplińska, "The potential role of cinnamon in human health," *Forests*, vol. 12, no. 5, pp. 1–17, 2021, doi: 10.3390/f12050648.
- [18] M. Stianto, Y. Peristiowati, and S. Farida, "The Benefits of Red Betel Leaf Extract for Perineal Wound Healing in BPM Rini District Kediri," *J. Qual. Public Heal.*, vol. 1, no. 2, pp. 58–66, 2018, doi: 10.30994/jqph.v1i2.18.
- [19] M. A. Suri, Z. Azizah, and R. Asra, "A Review: Traditional Use, Phytochemical and Pharmacological Review of Red Betel Leaves (*Piper Crocatum* Ruiz & Pav)," *Asian J. Pharm. Res. Dev.*, vol. 9, no. 1, pp. 159–163, Feb. 2021, doi: 10.22270/AJPRD.V9I1.926.
- [20] M. Anugrahwati *et al.*, "Extraction of Ethanolic Extract of Red Betel Leaves and Its Cytotoxicity Test on HeLa Cells," *Procedia Eng.*, vol. 148, pp. 1402–1407, 2016, doi: 10.1016/j.proeng.2016.06.569.
- [21] L. Widowati, L. HAndayani, and R. Mujahid, "The use of betel (*Piper betle*) leaves for maintaining the health of women and children at various ethnic groups in Indonesia," *Nusant. Biosci.*, vol. 12, no. 2, pp. 120–126, 2020, doi: 10.13057/nusbiosci/n120206.
- [22] S. D. Fitzmaurice, R. K. Sivamani, and R. R. Isseroff, "Antioxidant therapies for wound healing: A clinical guide to currently commercially available products," *Skin Pharmacol. Physiol.*, vol. 24, no. 3, pp. 113–126, 2011, doi: 10.1159/000322643.
- [23] P. Broz and V. M. Dixit, "Inflammasomes: Mechanism of assembly, regulation and signalling," *Nature Reviews Immunology*, vol. 16, no. 7. Nature Publishing Group, pp. 407–420, 01-Jul-2016, doi: 10.1038/nri.2016.58.
- [24] D. M. Haas, S. Morgan, K. Contreras, and S. Enders, "Vaginal preparation with antiseptic solution before cesarean section for preventing postoperative infections," *Cochrane Database Syst. Rev.*, vol. 2018, no. 7, 2018, doi: 10.1002/14651858.CD007892.pub6.
- [25] C. R. Duffy, J. Garcia-So, B. Ajemian, C. Gyamfi-Bannerman, and Y. W. Han, "A randomized trial of the bactericidal effects of chlorhexidine vs povidone-iodine vaginal preparation," *Am. J. Obstet. Gynecol. MFM*, vol. 2, no. 3, p. 100114, 2020, doi: 10.1016/j.ajogmf.2020.100114.
- [26] R. Danarti, . Suwardana, A. Budiyanto, and W. Wirohadidjojo, "The effect povidone-iodine on the wound healing process: A study on fibroblast populated collagen lattice (FPCL) model," *J. the Med. Sci. (Berkala Ilmu Kedokteran)*, vol. 46, no. 3, pp. 103–107, 2014, doi: 10.19106/jmedscie.004603201401.
- [27] N. Karimah, N. Khafidhoh, T. S. Hardjanti, and R. I. Hakim, "The Period of Perineal Wound Healing in Postpartum Mothers Between The Decoction Water Treatments of Bihanong Leaves with Red Betel Leaves," *GHMJ (Global Heal. Manag. Journal)*, vol. 3, no. 3, p. 107, 2019, doi: 10.35898/ghmj-33454.
- [28] P. L. Bigliardi, S. A. L. Alsagoff, H. Y. El-Kafrawi, J. K. Pyon, C. T. C. Wa, and M. A. Villa, "Povidone iodine in wound healing: A review of current concepts and practices," *Int. J. Surg.*, vol. 44, pp. 260–268, 2017, doi: 10.1016/j.ijsu.2017.06.073.
- [29] S. Wass, G. Albrektsen, M. T. Ødegård, M. Sand, and D. Austeng, "Antiseptic effect of low-concentration povidone-iodine applied with a depot device in the conjunctiva before cataract surgery," *Eye*, vol. 32, no. 12, pp. 1900–1907, 2018, doi: 10.1038/s41433-018-0198-9.
- [30] K. E. Johnson and T. A. Wilgus, "Vascular Endothelial Growth Factor and Angiogenesis in the Regulation of Cutaneous Wound Repair," *Adv. Wound Care*, vol. 3, no. 10, pp. 647–661, 2014, doi: 10.1089/wound.2013.0517.
- [31] H. Hong and X. Y. Tian, "The role of macrophages in vascular repair and regeneration after ischemic injury," *Int. J. Mol. Sci.*, vol. 21, no. 17, pp. 1–12, 2020, doi: 10.3390/ijms21176328.
- [32] Y. T. Tung, P. L. Yen, C. Y. Lin, and S. T. Chang, "Anti-inflammatory activities of essential oils and their constituents from different provenances of indigenous cinnamon (*Cinnamomum osmophloeum*) leaves," *Pharm. Biol.*, vol. 48, no. 10, pp. 1130–1136, 2010, doi: 10.3109/13880200903527728.
- [33] P. Supratim Mahapatra, Shivam Roy, Pushpita Chakraborty, Namrata Chakraborty, Utpalendu Paul and P. S. and M. B. Chatterjee, Subhadeep Banerjee, "CINNAMALDEHYDE, THE MAJOR COMPONENT OF CINNAMOMUM ZEYLANICUM, AFFECTS INFLAMMATORY PATHWAYS," *Angew. Chemie Int. Ed. 6(11)*, 951–952., vol. 11, no. 11, pp. 5788–5791, 2020, doi: 10.13040/IJPSR.0975-8232.11(11).5788-91.
- [34] X. Han and T. L. Parker, "Antiinflammatory Activity of Cinnamon (*Cinnamomum zeylanicum*) Bark Essential Oil in a Human Skin Disease Model," *Phyther. Res.*, vol. 31, no. 7, pp. 1034–1038, 2017, doi: 10.1002/ptr.5822.
- [35] S. F. Nabavi, A. Di Lorenzo, M. Izadi, E. Sobarzo-Sánchez, M. Daglia, and S. M. Nabavi, "Antibacterial effects of cinnamon: From farm to food, cosmetic and pharmaceutical industries," *Nutrients*, vol. 7, no. 9, pp. 7729–7748, 2015, doi: 10.3390/nu7095359.
- [36] R. B. Paiano, J. Bonilla, R. L. M. De Sousa, A.

- M. Moreno, and P. S. Baruselli, "Chemical composition and antibacterial activity of essential oils against pathogens often related to cattle endometritis," *J. Infect. Dev. Ctries.*, vol. 14, no. 2, pp. 177–183, 2020, doi: 10.3855/jidc.12076.
- [37] H. YS and N. L, "The accumulation of two neolignan in the leaves, stems, and flower of red betel (*Piper crocatum* Ruiz & Pav.)," *Int. Symp. Bioinformatics, Chemom. Metabolomics*, vol. 835, pp. 2–6, 2017, doi: 10.1088/1742-6596/835/1/012017.
- [38] K. Gurning, S. Lumbangaol, R. F. R. Situmorang, and S. Silaban, "Determination of phenolic contents and antioxidant activity test of ethanol extract of Sirih merah (*Piper crocatum* Ruiz & Pav.) leaves using the DPPH method," *J. Pendidik. Kim.*, vol. 13, no. 2, pp. 137–142, 2021, doi: 10.24114/jpkim.v13i2.26984.
- [39] B. Setiawan, I. Zarqya, S. Putro, and F. Khasanah, "The effect of red betel leaf's essential oil (*Piper Crocatum* Ruiz & Pav.) against Third Instar *Aedes aegypti* Larvae," *Pakistan J. Med. Heal. Sci.*, vol. 13, no. 4, pp. 1162–1164, 2019.
- [40] and S. B. Kai Lu, Madhavi Bhat, "Plants and their active compounds: natural molecules to target angiogenesis," *Angiogenesis*, vol. 19, no. 3, pp. 139–148, 2016, doi: 10.1007/s10456-016-9512-y.Plants.
- [41] A. Shedoeva, D. Leavesley, Z. Upton, and C. Fan, "Wound healing and the use of medicinal plants," *Evidence-based Complement. Altern. Med.*, vol. 2019, no. Figure 1, 2019, doi: 10.1155/2019/2684108.
- [42] A. Marchese *et al.*, "Antimicrobial activity of eugenol and essential oils containing eugenol: A mechanistic viewpoint," *Crit. Rev. Microbiol.*, vol. 43, no. 6, pp. 668–689, 2017, doi: 10.1080/1040841X.2017.1295225.