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Healing Effect of Wistar Rat Incision Wounds on Administration of Microemulsion Combination of Green and Red Betel Leaf Extracts

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Abstract: One natural ingredient that has the potential to heal wounds is betel leaf. This study aimed to analyze the healing effect of Incision wounds in Wistar rats when administering a microemulsion combination of green and red betel leaf extracts. This research begins with sample collection, drying process, and extraction. The extract was then formulated into emulgel as a microemulsion in three formulations. These three formulas, along with a positive control and a negative control, were tested on each Wistar rat incision wound. The results of the research showed that emulgel formula 3, which was tested on mice, had the best ability to heal wounds for an average of 2.5 days and kept the mice's body temperature normal; 36.6ºC. Formula 3 contains 5% South Kalimantan red betel, 5% South Kalimantan green betel, 5% Central Kalimantan red betel, and 5% Central Kalimantan green betel. This proves that the combination of formula 3 can heal wounds and body temperature in experimental mice. Further research is needed to find the best composition that produces faster, more complete wound healing effects and safely impacts experimental animals.
Keywords: Green betel; Incision wounds; microemulsion; red betel.

INTRODUCTION

Wounds can occur as a result of various human activities; wounds are a condition where part of the body's tissue is damaged so that the skin structure separates[1]. Wound Cuts are one of the most common injuries that happen in everyday life. The cut is damage or loss of tissue body due to sharp objects, where possible, causing bleeding and inflammation. There is a wound that can interfere with the activities of sufferers[2].

Data results Riskesdas Indonesia in 2013 and 2018 saw an increase in the prevalence of wounds from 8.2% to 9.2%[3]. The prevalence of wounds in Indonesia is relatively high as it stands, increasing traffic accidents[4]. Therefore, wound healing is necessary noticed. Wound healing is a process complex with stages, namely inflammation, proliferation, and maturation[5]. Improper wound healing can cause the wound to develop, leading to infection and prolonging the healing time of the wound[6]. Current wound healing uses chemical drugs, namely povidone iodine, but the use of these drugs can cause side effects, namely dermatitis or allergies[7]. Therefore, more alternative medicines must be practical and have minimum side effects when healing wounds.

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One natural ingredient with potential as a wound healing agent is betel leaf because it contains tannin, saponin and flavonoid compounds as well as other compounds that play a role, namely beta-phenol and chavicol[8]. The general public uses betel. There are two types of betel: green betel and red betel. This plant is widely distributed in tropical and subtropical areas in various parts of the world[9]. Betel plants grow in tropical Asia to East Africa, spreading almost throughout Indonesia, Thailand, Malaysia, India, Sri Lanka and Madagascar[10]. Betel is a medicinal plant which is the potential and is known empirically to have various healing properties for disease[9]. The community uses green betel leaves for treatment to stop bleeding, itching, and canker sores and cure diseases caused by bacterial or fungal infections[11-12]. Apart from that, betel root is also used as a contraceptive drug in women[13].

Literature review In several previous studies, green betel leaf extract (*Piper betle* L.) in a concentration of 2-10% is effective in wound healing, significantly influencing the inflammation and proliferation processes[14]. Green betel leaf extract with a concentration of 7% in spray has been proven to heal wounds in mice and has comparable activity to oxoferin[15]. Research on male white rats (Rattus norvegicus) shows that ointment Ethanol extract from betel leaves can speed up wound healing[16].

Previous research has been carried out on betel leaves as a wound healer. However, it is still rare for research to develop preparations with variations in the composition of green betel leaves and red betel leaves for wound healing; in addition, according to Akter et al. on 2014, compounds The active chemicals contained in betel leaves are influenced by geographic area and the environment[17]. This research combines the active compounds of betel leaves originating from two provinces in Indonesia, namely Central Kalimantan and South Kalimantan. The microemulsion combination of green and red betel leaf extracts was tested on cuts from the Wistar rat.

MATERIALS AND METHODS

Experimental Protocol

The test animal used was a female Wistar rat. A total of 20 test animals were divided into 5 groups, namely positive control group, negative control group, formula 1 group, formula 2 group, formula 3 group. Each group received treatment in the form of 1) The positive control group was given a 2.5% chloramphenicol emulgel formula, 2) The negative control group was given an emulgen formula without active ingredients, 3) The formula 1 group was given an emulgel containing 10% South Kalimantan green betel and 10% Central Kalimantan green betel. %, 4) Formula 2 group was given emulgel containing 10% red betel from South Kalimantan and 10% red betel from Central Kalimantan, 5) Formula 3 group was given emulgel containing 5% green betel from South Kalimantan, 5% green betel from Kalimantan Central Kalimantan, red betel 5% from South Kalimantan 5%, red betel from Central Kalimantan 5%. Before testing began, all test animals had their body temperature measured, and blood samples were taken for haematology testing. Next, each test animal was given a wound 1 cm long on the mouse, then exposed to bacteria orally using a probe. After 60 minutes, all treatment groups were each given the emulgel formula that had been made on the wound area of the mice. The formula is given twice a day for three days. On the fourth day, the test animals had their body temperature checked and their blood taken, followed by a haematology test. Body temperature and haematology results of mice before and after treatment were compared.

This research proposal has been examined by the Research Ethics Commission of Muhammadiyah University of Banjarmasin, as stated in certificate number 540/UMB/KE/VII/2023.

Making Simplisia and Extracting Betel Leaves

Green and red betel leaves were collected from South Kalimantan and Central Kalimantan, so four types of leaves were obtained. The leaves are then dried using an oven at a temperature of no more than 60ºC. The dried leaves are then blended to produce simplicia powder. The extraction process includes soaking the leaf powder using 70% ethanol solvent. The extraction process was carried out 3 x 24 hours with solvent changes every 24 hours[18]. The solvent from soaking the leaf powder is collected and filtered; the solvent is evaporated until a dry extract is obtained.

The extract obtained organoleptically is solid, black in colour, has a distinctive odour, and is slightly bitter. The solid form is obtained from solvent evaporation, so the betel leaf juice remains solid[19]. Black is the colour commonly used in extracts because the colouring substances in the leaves are generally not active compounds, so they are usually not absorbed into the extract. The distinctive aroma is obtained from the essential oils still found in betel leaf extract. The bitter taste of an extract is expected because the active ingredients generally taste bitter, like the active ingredients in conventional medicines. Formulation techniques will easily cover this bitter taste in the dosage form[20].

Making Emulgel Formula

The formula is made in emulgel dosage form in five formulas. The formula consists of negative control, positive control, formula 1, formula 2, and formula 3. The negative control emulgel only consists of emulgen-forming ingredients without active ingredients. The positive control emulgel consisted of emulgel containing chloramphenicol. Emulgen Formula 1, Formula 2, and Formula 3 contain betel leaf extract. The composition of the emulgel is in Table 1, and the results of the emulgel formed are presented in Figure 1.

Table 1. Emulgel Preparation Formulation

Material	**Concentration (%)**				
	Negative Control	**Positive Control**	**F1**	**F2**	**F3**
South Kalimantan Green Betel	-	-	10	-	5
South Kalimantan Red Betel	-	-	-	10	5
Central Kalimantan Green Betel	-	-	10	-	5
Central Kalimantan Red Betel	-	-	-	10	5
Chloramphenicol	-	2.5	-	-	-
Chitosan	2	2	2	2	2
Propylene glycol	5	5	5	5	5
Carbopol	1	1	1	1	1
Tween 80	0.6	0.6	0.6	0.6	0.6
Span 80	2,3	2,3	2,3	2,3	2,3
VCO	5	5	5	5	5

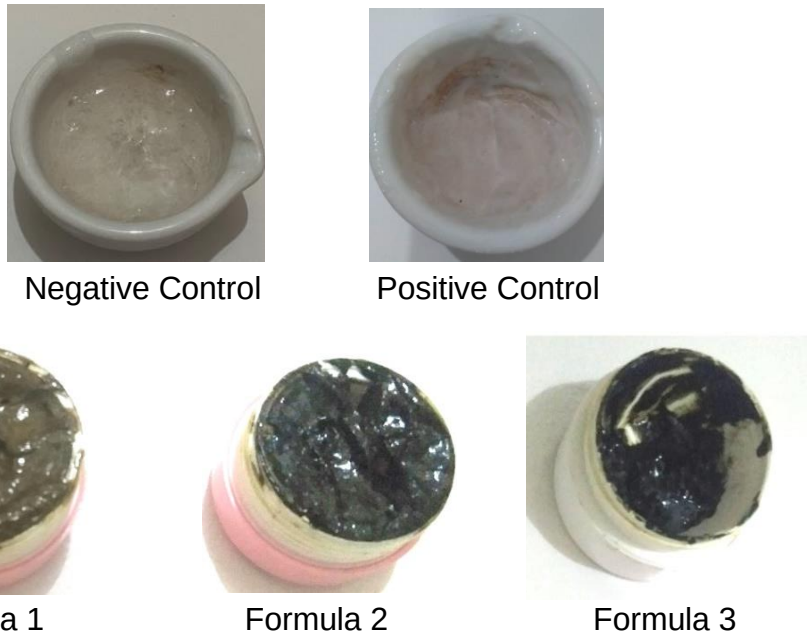

Figure 1. Emulgel Formula from Research Results

The emulgel formed is visually apparent for the negative control formula because it only contains gel-forming ingredients, which are evident in colour. The positive control emulgel formula is milky white because it has chloramphenicol, which is basically white. Formula 1 has a slight green appearance because this formula contains green betel leaf extract, which is visible when formulated in emulgel form. In formula 2 and formula 3, there is no significant colour difference visually because the emulgel is a mixture with a uniform concentration of the four betel leaf extracts. According to the literature, the emulgel formed has a colour that matches the colour of the active ingredient[21].

Mice GroupTreatment

The adaptation process in rats is essential before treatment is given. Adaptation is carried out for at least 7 days to make the mice feel comfortable[22]. This research carried out a rat adaptation process for one week. Testing was carried out on 5 rat groups: positive control group, negative control group, formula 1 group, formula 2 group, and formula 3 group. One day before the wound was applied slashed, the rat hair on his back was shaved until smooth. The shaved area was cleaned using 70% alcohol, and then the rat was rested for 1 day. The next day, the incision was made using a scalpel. Wounds caused by the length of the wound are 2 cm, and the depth is ± 0.2 cm[23]. Each wound was given an emulgel formula according to the treatment group, which was delivered twice a day for three days. Observation of incision wound healing was carried out visually every day. Observations included body temperature and wound healing time; the mice's body temperature was measured before and after being treated on the 4th day, and the wound healing time was on the day that >30% wound healing was seen. The percentage

of wound healing is calculated by the wound healing area divided by the initial wound area multiplied by 100%.

RESULTS AND DISCUSSION

The test results showed that all rats before treatment in the five groups had average body temperatures, namely 36.65 – 37ºC. After treatment, the negative control group's average body temperature increased to 38.45ºC. This was probably because the mice had an infection due to not being treated. In the formula 1, formula 2 and formula 3 groups, there were differences in the healing time of cuts; formula 1, 2, 3, positive control, and negative control had an average wound healing (in days) of 4.75; 4; 2.5; 2.25, and 6.25. So, formula 3 has the best ability to heal wounds because it is not too different from the positive control.

Table 2. Results of Wound Healing and Body Temperature of Rats in the Treatment Group

No	Group	Replication	Wound Closure (Days)	Temperature (ºC)	
				Before	After
1	Formula 1	1	4	36.7	37.5
		2	5	36.8	37.2
		3	5	37.2	37
		4	5	37.1	36.8
		Average	4.75	36.95	37,125
2	Formula 2	1	4	36.4	37.1
		2	4	36.4	36.6
		3	4	36.8	36.4
		4	4	37.1	36.9
		Average	4	36,675	36.75
3	Formula 3	1	3	36.8	36.6
		2	2	36.5	36.5
		3	2	36.6	36.5
		4	3	36.7	36.8
		Average	2.5	36.65	36.6
4	Positive Control	1	2	37.1	36.5
		2	2	37.2	36.8
		3	2	36.8	36.9
		4	3	36.9	37
		Average	2.25	37	36.8
5	Negative Control	1	6	36.6	38.5
		2	6	37.2	38.6
		3	7	37.1	38.3
		4	6	36.9	38.4
		Average	6.25	36.95	38.45

The research showed that Formula 3 had the best ability to heal wounds with an average of 2.5 days (Table 2). Another study explained similar results, stating that topical administration of green betel leaf extract was proven to heal wounds in male Wistar rats[24].

Research by Fannani et al (2014) also supports these results, stating that betel leaf ethanol extract ointment is able to accelerate the wound healing process of male white rats (*Rattus norvegicus*)[16]. Healing of wounds in mice can occur because of the active substances in betel leaves, which play a role in wound healing. The compounds which play a role include betaphenol and chavicol[8]. The most widely used ingredient in betel leaves is the leaf because it contains 4.2 essential oils. % and most of its components consist of beta phenol, which acts as an antibacterial agent[25]. Green betel leaves contain several other ingredients such as steroids, tannins, flavonoids, saponins, phenols, alkaloids, coumarins, and emodins[26].

Betel leaves contain vitamin C, which can increase the stimulation of collagen formation by cell fibroblasts[27-28]. Vitamin C derivative, magnesium ascorbyl phosphate, can increase cell proliferation and stimulate collagen synthesis because collagen proliferation is essential in the improvement process network[29]. Betel leaves also contain hydroxychavicol, an anti-inflammatory compound that accelerates wound healing. The compound hydroxychavicol suppresses TNF expression in human neutrophils[30-31]. Betel leaves have a content of saponin compounds that function as an antioxidant, antifungal, and antimicrobial. Antioxidant activity proved the ability to form hydroperoxide intermediates, which prevent bio-molecular damage by free radicals[32-33].

The limitations of this research are the need for variations in the microemulsion formula combining green and red betel leaf extract and the wound observation time, which lasted only a short time. Further research is needed to find the best composition that produces faster, more complete wound healing effects and safely impacts experimental animals.

CONCLUSION

The research showed that emulgel formula 3 had the best ability to heal wounds with an average of 2.5 days. Formula 3 contains 5% South Kalimantan red betel, 5% South Kalimantan green betel, 5% Central Kalimantan red betel, and 5% Central Kalimantan green betel. Further research is needed to find the best composition that produces faster, more complete wound healing effects and safely impacts experimental animals.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

REFERENCES

1. Buulolo, AJ, and Syamsul, D. Formulation of Aloe Vera (*Aloe Vera* L.) Gel Preparation as a Wound Medicine. World Journal of Pharmacy. 2016;1(1):1-6.

2. Nurihardiyanti, N., Werawati, A., Kasumawati, F., and Ahaditama, R. Pharmacological Test of Toxic Jatropha Curcas L. Plant Patch Plaster for Healing Cut Wounds From Various Literature. In ongoing proceedings: National Seminar on Research Results and Community Service. 2020;1(1):1373-1380.
3. Ministry of Health of the Republic of Indonesia. National Riskesdas Report 2018. Publishing Institution Health Research and Development Agency (LPB): Jakarta. 2019.
4. Mahyudin F, Edward M, Basuki MH, Basrewan Y and Rahman A. Modern and Classic Wound Dressing Comparison In Wound Healing, Comfort And Cost. Nursing Journal. 2020;15(1):31-36.
5. Mohanty, C., and Sahoo, S. K. Curcumin and Its Topical Formulations For Wound Healing Applications. Drug Discovery Today. 2017;22(10):1582-1592.
6. Suharto, IPS, Ramayanti, ED, Mei Yunalia, E., and Ulfa, N. Betel Leaf Extract's Effect (Piper batle Linn) On The Healing Process Of Incision Wounds In Rats (Rattus norvegicus). Indonesian Journal of Nursing Education (JPKI). 2021; 7(2):123-128.
7. Rahmawati, I. Differences in the Effects of Wound Treatment Using Crushed Chinese Petai (*Leucaena glauca Benth*) Leaves and 10% Povidone Iodine in Accelerating Clean Wound Healing in Marmots (*Cavia porcellus*). Wiyata Journal. 2014;1(2):227-234
8. Lien LT, Tho NT, Ha DM, Hang PL, Nghia PT, Thang ND. Influence of Phytochemicals In Piper Betle Linn Leaf Extract On Wound Healing. Burns Trauma. 2015; 3(23).
9. Chakraborty D and Shah B. The antimicrobial, antioxidative and antihemolytic activity of Piper betel leaf extracts. International Journal of Pharmacy and Pharmaceutical Sciences. 2011; 3(3):192-199.
10. Gultom FKB, Nababan J, Sinambela TM, Harizka T, and Rahmatsyah R. Ethanol Absorbance Test on Green Betel Leaves (*Piper betle* L.) using the UV-Vis Spectrophotometry Method. Einstein (e-Journal). 2017; 5(2): 20-24.
11. Qonitah F and Ahwan. Antioxidant activity and total phenolic content of the polar isolate of the hexane fraction of ethanol extract of betel leaves (*Piper betle* L.). Pharmaceutical Journal. 2018; 7(2):42-46.
12. Caburian A.B and Osi MO. Characterization and evaluation of the antimicrobial activity of the essential oil from the leaves of *Piper Betle* L. E-International Scientific Research Journal. 2010; 2(1):2-13.
13. Hoque, M.M., Rattila, S., Shishir, M.A., Bari, M.L., Inatsu, Y., and Kawamoto, S. Antibacterial activity of ethanol extract of betel leaf (*Piper betle* L.) against some food-borne pathogens. Bangladesh Journal of Microbiology. 2011; 28(2):58-63.
14. Darmawan, A., Yusuf, S., Tahir, T., and Syahriyani, S. Betel leaf extract efficacy on wound healing: a systematic review. STRADA Health Scientific Journal. 2021; 10(1):526-536
15. Sikumbang, IM, Nurani, LH, Yuliani, S., and Edityaningrum, CA. The Effect Of Betel Leaf Extract Spray On Histopathology Of Wound Healing. Journal of Scientific and Practical Pharmacy. 2022; 8(2):162-169
16. Fannani, Muhammad Z., and Taufiq Nugroho. Effect of Ethanol Extract Ointment of Betel Leaves (*Piper Betle*) on Healing of Incision Wounds in Male White Rats (Rattus Norvegicus). Indonesian Journal of Medicine and Health. 2014; 6(1):20-27.

17. Akter, K.N., Karmakar, P., Das, A., Anonna, S.N., Shoma, S.A., and Sattar, M.M. Evaluation of antibacterial and anthelmintic activities with total phenolic contents of Piper betel leaves. Avicenna Journal of Phytomedicine. 2014; 4(5):320-329.
18. Subiyandono & A. Nurhasanah. Antioxidant Activity Test of Breadfruit Leaves (*Artocarpus altilis*), Jackfruit Leaves (*Artocarpus heterophyllus*), and Cempedak Leaves (*Artocarpus champeden*) using the DPPH Method. Journal of Health. 2015; 10:21-29.
19. Kamarudin, NA, M. Markom & J. Latip. Effects of Solvents and Extraction Methods on Herbal Plants *Phyllanthus niruri*, *Orthosiphon stamineus* and *Labisia pumila*. Indian Journal of Science and Technology. 2016; 9:1-5.
20. Rizki, MI, AK Sari, D. Kartika, A. Khairunnisa & Normaidah. Determination of Total Phenolic Content and Test of Antioxidant Activity of Fractions from Ethanol Extract of Cempedak Leaves (*Artocarpus integer*) using the DPPH Method. Media Pharmaceutica Indonesiana. 2022; 4:168-178.
21. Khadijah, AM Jayali, S. Umar & I. Sasmita. Determination of Total Phenolic and Antioxidant Activity of Ethanolic Extract of Daunsamama (*Anthocephalus macrocephalus*) from Ternate, North Maluku. Mulawarman Chemistry Journal. 2017; 15:11-18.
22. Kurniawati, D. Antibacterial Activity Test of Propolis Trigona spp. Origin of High Hills in the Sprague-Dawle White Rat. J. Prog. Kim. Si. 2011; 1(1):23-31.
23. Aini, DN, Ningsih, D., & Pramukantoro, GE. Effectiveness Test of Patch Green Betel Leaf Extract (*Piper betle* L.) on Healing of Rabbit (*Oryctolagus cuniculus*) Back Wounds: Effectiveness Test of Patch Green Betel Leaf Extract (*Piper betle* L.) on Healing of Rabbit (Oryctolagus cuniculus) Back Wounds. Journal of Science And Health. 2023; 5(5), 837–849. https://doi.org/10.25026/jsk.v5i5.1942
24. Palumpun, EF, Wiraguna, AA, and Pangkahila, W. Topical administration of betel leaf extract (*Piper Betle*) increases epidermis thickness, fibroblasts, and collagen in wound healing in male Wistar rats (*Rattus norvegicus*). eBiomedicine. 2017; 5(1):1-7.
25. Dwianggraini, R., Pujiastuti, P., and Ermawati, T. Differences in antibacterial effectiveness between red betel leaf extract (Piper scrotum) and green betel leaf extract (Piper betle L.) against Porphyromonas gingivalis. Stomatognathic-Journal of Dentistry. 2013; 10(1): 1-5
26. Patil, RS, Harale, PM, Shivangekar, KV, Kumbhar, PP, and Desai, RR. Phytochemical potential and in vitro antimicrobial activity of Piper betle Linn. leaf extracts. Journal of Chemical and Pharmaceutical Research. 2015; 7(5):1095-1101.
27. Pradhan D, Suri KA, Pradhan DK, Biswasroy P. Golden Heart of the Nature: Piper betle L. J Pharmacogn Phytochem. 2013; 1(6):147-167.
28. Tiedtke J. Stimulation of Collagen Production in Human Fibroblasts. Cosmetic Science Technology. 2007.
29. Zulaechah. Differences in Healing Speed of Cut Wounds Between Using Snail Slime (*Achatina fulica*) and Povidone Iodine 10% in the Treatment of Cut Wounds in Mice (*Mus musculus*). Thesis. Nursing Science Study Program, Faculty of Medicine and Health Sciences, Muhammadiyah University of Yogyakarta. 2010.

30. Sharma S, Khan IA, Ali I, Ali F, Kumar M, Kumar A. Evaluation of The Antimicrobial, Antioxidant, and Antiinflammatory Activities of Hidroxychavicol for Its Potential Use As an Oral Care Agent. Antimicrob Agents Chemother. 2009; 53(1):216-222.
31. Bhattacharya S, Banerjee D, Bauri AK, Chattopadhyay S, Bandyopadhyay SK.. Healing Property of The Piper Betel Phenol, Allylpyrocatechol Against Indometachin-Induced Stomach Ulceration and Mechanism of Action. World J Gastroenterol 2007; 13(2):3705-3713.
32. Francis G. Kerem Z, Makar HPS, Becker K. The Biological Action of Sapoinins in Animal Systems: A Review. Br J Nutr. 2002; 88:587-605.
33. Prabu MS, Muthumani M, Shagirta K. Protective Effect of Piper betle Leaf Extract Against Cadmium-Induced Oxidative Stress and Hepatic Dysfunction in Rats. Saudi J Biol Sci. 2012; 10:1-11.