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Exploration of the healing effect of red betel leaf essential oil ointment on incision wounds in wistar rats (Rattus Norvegicus)

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Abstract
The red betel plant (Piper crocatum) is abundant in medicinal compounds, primarily found in its leaves, including essential oils, alkaloids, saponins, tannins, and flavonoids. Chromatogram results confirm the presence of these compounds, providing a robust scientific basis for using red betel in traditional medicine. Renowned for its antiseptic and antibacterial properties, red betel's chemical elements contribute to its treatment efficacy. This study, conducted in January 2024, aimed to assess the effectiveness of ointment derived from the essential oil of red betel leaves (Piper crocatum Linn.) in healing wounds in Wistar rats (Rattus norvegicus). Using an experimental approach with a Pre-test and Post-test group-only control design, the study involved 25 randomly selected white male rats divided into five groups (four treatment groups and one control group), each consisting of five rats. The essential oil Saleb of red betel leaf extract (Piper crocatum Linn.) contains bioactive compounds like alkaloids, flavonoids, saponins, and tannins, contributing to its role in wound healing. The optimal concentration for recovery of cut wounds in white rats is 9% v/v. The highest healing percentage on day 14 was observed in the positive control (Bioplacenton®) at 95%, followed by the 9% v/v extract with a 90% healing rate. The essential oil Saleb preparation of red betel leaf extract (Piper crocatum Linn.) demonstrates efficacy comparable to Bioplacenton® in recovering cut wounds in rats.

Keywords: Red betel plant, medicinal compounds, wound healing, essential oil saleb, experimental study

Introduction
The red betel plant (Piper crocatum) is a valuable natural resource abundant in medicinal compounds, particularly within its leaves. The leaves of red betel encompass an array of phytochemical compounds, such as volatile oils, alkaloids, saponins, tannins, and flavonoids. Chromatogram results underscore the presence of essential oils, tannins, polevenolad compounds, and flavonoids among the phytochemical constituents. Furthermore, the chemical composition of red betel leaves includes hydroxycavicol, chavicol, capitol, carvacrol, eugenol, p-simen, cineol, caryophyllene, cadimen estragole, terpenes, and phenyl prostanoids. This comprehensive information is a robust scientific foundation supporting the potential utilization of red betel leaves in traditional medicine, wherein these compounds offer diverse health benefits (Damarini *et al*., 2013) [5]. Notably, the chemical elements present in the red betel plant, particularly in its leaves, have proven helpful in treatment, contributing to its widespread use in traditional medicine. Extracts from Piper crocatum or red betel leaves are known to contain antiseptic and antibacterial chemicals (Zubier *et al*., 2010) [14]. In the Indonesian context, betel nut is renowned for its medicinal properties, serving as an antimicrobial, antihypertensive, antiallergic, anti-inflammatory, hepatoprotective, antioxidant, and antidiabetic agent (Windono, 2016). As emphasized by Tomagola *et al*. (2016), natural antioxidants are commonly derived from spices, herbaceous plants, fruits, vegetables, and grains.

Red betel (Piper crocatum Ruiz & Pav.) stands out as one of the herbaceous plants in Indonesia with notable applications as a natural antioxidant, as highlighted by Tomagola (2016) and Muhammad A'tourrohman & Malia Ulfah (2020) [7]. Among its chemical constituents, red betel leaves, specifically, have become widely recognized for their therapeutic potential (Diniatik, 2011) [6]. The phytochemical compounds in red betel leaves encompass essential oils, alkaloids, saponins, tannins, and flavonoids, as elucidated by Beon & Leki (2017) [4] and Ulviani *et al*. (2016). Natural wound healing involves distinct phases, including inflammation, proliferation, and maturation or remodeling. In the inflammatory phase, crucial elements like hemostasis come into play to naturally stop bleeding when a wound occurs. The release of histamine and other mediators facilitates clotting through platelets, thrombin, and fibrin. Subsequently, the proliferative phase witnesses the formation of new blood vessels, collagen tissue deposition, and fibroblast and epithelial cell infiltration, leading to granulation tissue formation. The remodeling phase, commencing around 2-3 weeks post-injury, involves the transformation of granulation tissue into scar tissue, with recapitulation determining the strength and arrangement of the connected cell tissue and new epithelium (Sentat & Permatasari, 2015) [8].

The phytochemical composition of red betel leaves, including essential oils, alkaloids, saponins, tannins, and flavonoids, further accentuates its medicinal attributes (Ulviani *et al*., 2016). The antioxidants found in red betel are renowned for combating diseases triggered by free radicals. Notably, betel leaves boast a 4.2% volatile oil content, predominantly comprising betephenol, an isomer of Eugenol allypyrocatechine, Cineol methyl eugenol, and caryophyllene (sesquiterpene), chavicol, kavibekol, estragole, and terpinene. Additionally, the essential oils in red betel exhibit antibacterial properties, aiding in preventing infections in burn wounds. The flavonoid content contributes to its effectiveness as an anti-inflammatory agent, as supported by research comparing red betel and green betel leaf extracts in inhibiting the growth of Escherichia coli (Syahrinastiti, 2015; Syahrinastiti *et al*., 2015) [9, 9]. Given these attributes, this study aims to evaluate the efficacy of an ointment formulated from red betel leaf essential oil in wound healing in Wistar rats (Rattus norvegicus).

Research Methods

The research conducted in January 2024 is an experimental study employing a Pre-test and Post-test group-only control design approach. The study involved a sample of 25 randomly selected white male rats, which were then divided into five groups comprising four treatment groups and one control group, with each treatment group consisting of 5 rats. The materials utilized in the study encompassed alcohol, aluminum foil, distilled water, red betel, 96% ethanol, rat test animals (mus musculus), sterile gauze, Whatman filter paper, methylparaben, petroleum ether, plaster, propylene glycol, gloves, and triethanolamine. The tools employed included glassware (pyrex®), an autoclave, a maceration vessel, a blender (Maspion®), a porcelain cup, a caliper (Tricle brand®), an oven, tweezers, a rotavapor (Heidolf®), an iron spoon, an analytical balance (Precisa®), and a water bath.

Calculation of Red Betel Leaf Oil Yield (*Piper crocatum linn.*)

The calculation of the yield of Red Betel Leaf Oil (piper crocatum linn.) (Amaliah *et al*., 2019).

$$\text{Yield (\%)} = \frac{\text{Red Betel Leaf Essential Oil x 100\%}}{\text{Sample Period of Red Betel Leaf}}$$

Observations were made regarding changes in the wound and the size of the damage in the treated area. Data analysis using statistical data tests including;

a. Normality Test
b. ANOVA test to determine the effectiveness of ethanol extract of Red Betel Leaf (piper crocatum linn.) and Bioplacenton® on rat back incision wound healing.

Results

Table 1 presents the chemical composition of Red Betel Leaf extract (Piper crocatum Linn.), revealing the presence of alkaloid, flavonoid, saponin, and tannin compounds. The alkaloid test yielded distinctive results, manifesting as a red-brown precipitate when exposed to the Dragendorff reagent, a white deposit upon the addition of the Mayer reagent, and a brown residue during the Bouchardt test (Beon & Leki, 2017) [4] (Abdullah Yeni, 2015) [1].

Tabel 1: Phytochemical Screening of Red Betel Leaf (piper crocatum linn.)

Test	Result	Description
Alkaloid	Red-brown precipitate	(+)
	White precipitate	(+)
	Brown precipitate	(+)
Flavonoid	The red color in the amyl alcohol layer	(+)
Saponin	Permanent foam	(+)
Tanin	Blackish green color	(+)

Table 2: Inhibition percentage data of Red Betel Leaf extract (piper crocatum linn.) against DPPH

Extract Concentration (ppm)	Absorbance Extract	Absorbance Control	Inhibisi (%)
3	0.322	0,544	56.12
6	0.289	0,544	57.48
9	0.244	0,544	57.86
12	0.135	0,544	59.10

Table 2 reveals that the absorbance of DPPH by Red Betel Leaf extract (Piper crocatum Linn.) exhibits a decrease corresponding to an increase in extract concentration. The experiment involves assessing the inhibitory activity of the section at various concentrations (3 ppm, 6 ppm, nine ppm, and 12 ppm). The absorbance values fluctuated, peaking at three ppm concentration (0.322) and reaching the lowest point at 12 ppm concentration (0.135), while the control's absorbance remained constant at 0.544 across all engagements. The percentage inhibition demonstrated an upward trend with increasing extract concentration, culminating at 12 ppm with an inhibition level of 59.10%. These findings underscore the significant impact of extract concentration on the inhibition process, with the highest concentration (12 ppm) yielding the maximum inhibitory effect in this experimental context.

In addition to inhibiting bacterial growth, Red Betel Leaf extract (Piper crocatum Linn.) also exhibits anti-fungal properties. The petroleum ether, ethyl acetate, and chloroform extracts of Red Betel Leaf demonstrated anti-fungal activity against Candida albicans and C. krusei, with the petroleum ether extract displaying superior anti-fungal efficacy compared to other solvents (Devi *et al*., 2015). The bioactivity of Red Betel Leaf (Piper crocatum Linn.) is attributed to essential oil constituents such as eucalyptol, limonene, δ-3-care, and methyl-cinnamate (Majumder *et al*., 2014).

The primary volatile oils identified in Red Betel Leaf encompass α-pinene, β-myrcene, limonene, (Z)-β-ocimene, (E)-β-ocimene, linalool, citronellal, α-terpineol, β-citronellol, neral, linalool acetate, geraniol, geranial, citronellyl acetate, (Z)-β-farnesene, A sesquiterpene, cubelol, (Z)-isoelemicene, A, (Z)-asarone, and (E)-asarone. The essential oils present in Red Betel Leaf demonstrate bioactivity, serving as anti-cancer and antimicrobial agents. The optimum concentration of Red Betel Leaf (piper crocatum linn.) extract was at 9% v/v, with the wound remaining 0.2 cm long. For more details, it can be seen in the table 4:

Table 3: Changes in wound length with various concentrations of Red Betel Leaf extract (Piper crocatum linn.)

Days	Change in Wound Length (cm)				
	Concentration 3%	Concentration 6%	Concentration 9%	Concentration 12%	Bioplacenton

1	2	2	2	2	2
3	1.8	1.7	1.7	1.7	1.7
5	1.7	1.4	1.4	1.4	1.3
7	1.4	1.1	1.1	1.2	0.9
9	1.2	0.8	0.8	1	0.7
11	1	0.5	0.5	0.8	0.4
14	0.8	0.3	0.2	0.5	0.1

The provided data detailing the evolution of wound length over time within groups subjected to distinct treatments (3%, 6%, 9%, and 12% concentrations of Red Betel Leaf Essential Oil Ointment and Bioplacenton®) exhibited diverse trends. Initially, on day 1, all groups presented identical wound lengths of 2 cm. However, throughout the observation period, fluctuations in wound length were observed in each group. By day 3, the 6% and 9% concentration groups displayed a relatively more significant reduction in wound length than the other groups. On day 7, the 12% and Bioplacenton® concentration groups demonstrated a swifter decrease in wound length. By day 14, the Bioplacenton® group exhibited the most petite wound length, succeeded by the 6% concentration group. These findings underscore the potential efficacy of Red Betel Leaf Essential Oil Ointment in facilitating wound healing, with the 9% concentration yielding a particularly favorable response.

Efektifitas Ekstrak ekstrak Daun Sirih Merah (*piper crocatum linn.)* Terhadap Penyembuhan Luka Sayat

It appears that the table containing the results of the ANOVA test is missing from your input. Please provide the relevant data or table. I would be happy to assist you in interpreting the results and drawing conclusions regarding the effectiveness of different concentrations of Red Betel Leaf extract (Piper crocatum Linn.) on wound healing.

Table 4: Test Results of the Effect of Red Betel Leaf Extract (Piper crocatum linn.) on Wound Healing

ANOVA						
		Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Red Betel Leaf Extract	Between Groups	5.622	3	3.451	7.125	.010
	Within Groups	36.367	22	.260		
	Total	45.156	25			

The Analysis of Variance (ANOVA) was employed to assess the statistical significance of differences in group means concerning the impact of Red Betel Leaf Extract. The obtained ANOVA results indicate a substantial distinction among the tested groups, as evidenced by an F value of 7.125 and a significance level of 0.010. This signifies that the observed variation in the effect of Red Betel Leaf Extract within these groups is not likely due to random chance. Consequently, it can be deduced that noteworthy differences exist in the response to Red Betel Leaf Extract across the tested groups, underscoring its potential impact within the scope of this study (Nuryadi, *et al*. 2017).

Effectiveness of Bioplacenton® on Incision Wound Healing

The Anova test can review the effect or effectiveness of Bioplacenton® on wound healing. The results of the test can be seen in the following table:

Table 5: Test Results of the Effect of Bioplacenton® (positive control) on Wound Length

ANOVA					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	12.785	3	1.114	25.166	.014
Within Groups	1.612	22	.045		
Total	11.838	25			

The Analysis of Variance (ANOVA) results are in Table 5. indicate the significant impact of Bioplacenton® administration (used as a positive control) on wound length, with an F value of 25.166 and a significance level (Sig.) of 0.014. This suggests a statistically significant difference in the means of groups receiving Bioplacenton®, reinforcing its efficacy in wound healing (Nuryadi *et al*., 2017). Bioplacenton® is a topical antibiotic gel containing 10% ex-bovine placenta extract and 0.5% neomycin sulfate, known for stimulating tissue formation and preventing bacterial infections in wounds (Kalbemed, 2013). The use of placenta extract in wound healing is supported by its rich content of bioactive molecules with anti-inflammatory, antianaphylactic, antioxidant, antimelanogenic, moisturizing, and collagen-forming properties (Park, 2010; Cho *et al*., 2006).

Luka dapat diklasifkasikan berdasarkan sifat, struktur anatomis, proses penyembuhan, lama penyembuhan, serta kedalaman dan luasnya luka (Kartika, 2015; Thomas, 2010). Berdasarkan sifatnya, luka dibagi menjadi luka abrasi, kontusio, insisi, laserasi, penetrasi, puncture, sepsis, dan lain-lain (Kartika, 2015). Berdasarkan struktur anatomisnya, luka diklasifikasikan menjadi luka superfisial yang meliputi bagian superfisial kulit yaitu epidermis (terkadang dapat sampai ke lapisan atas dermis), luka partial thickness yang melibatkan lapisan epidermis dan dermis, serta luka full thickness yang melibatkan seluruh lapisan kulit dari mulai epidermis, dermis, lapisan lemak, fascia, bahkan sampai ke tulang (Dealey dan Cameron, 2006; Kartika, 2015).

Berdasarkan proses penyembuhannya, luka dapat diklasifikasikan menjadi tiga, yaitu (Kartika, 2015) yakni penyembuhan luka primer, penyembuhan luka sekunder dan Delayed Primary Healing. Pada proses penyembuhan primer tepi luka bisa menyatu kembali, permukaan bersih, dan tidak ada jaringan yang hilang. (Kartika, 2015). Penyembuhan primer terjadi bila luka segera diupayakan bertaut, biasanya dengan bantuan jahitan. Jaringan granulasi yang dihasilkan sangat sedikit, dan biasanya jaringan parut yang terbentuk pada proses ini lebih halus dan kecil (Hasibuan, *et al*., 2010; Morison, 2004). Penyembuhan sekunder terjadi ketika tepi kulit terpisah jauh dan sebagian jaringan hilang. Proses penyembuhan berlangsung mulai dari pembentukan jaringan granulasi di dasar luka dan sekitarnya (Dealey dan Cameron, 2006; Kartika, 2015). Jaringan granulasi yang terdiri atas kapiler-kapiler darah baru yang disokong oleh jaringan ikat terbentuk di dasar luka dan sel-sel epitel melakukan migrasi ke pusat permukaan luka (Morison, 2004). Daerah permukaan luka menjadi lebih kecil akibat suatu proses yang dikenal sebagai kontraksi dan jaringan ikat disusun kembali hingga membentuk jaringan yang bertambah kuat. Pada penyembuhan sekunder akan meninggalkan jaringan parut yang nyata, bahkan dapat terbentuk jaringan parut keloid (Morison, 2004).

Wounds can be categorized based on their characteristics, anatomical structure, healing process, duration, and the depth and size of the injury (Kartika, 2015; Thomas, 2010). Regarding features, wounds are classified as abrasions, contusions, incisions, lacerations, penetrations, punctures, sepsis, and other types (Kartika, 2015). Anatomically, wounds are grouped into superficial, partial-thickness, and full-thickness wounds (Dealey and Cameron, 2006; Kartika, 2015). The wound-healing process involves primary, secondary, and delayed primary healing (Kartika, 2015). Primary healing occurs when the wound edges can be brought together without tissue loss, typically with sutures. Secondary recovery occurs when the skin edges are distant, forming granulation tissue and contraction, resulting in visible scars, including keloids. Delayed wound healing is employed for contaminated or poorly demarcated wounds, starting with cleaning and excision before suturing after a few days (Hasibuan *et al*., 2010; Kartika, 2015).

Wounds arising from trauma or temperature changes can manifest as cuts, punctures, lacerations, abrasions, or burns (Sjamsuhidajat, 2010). The intricate wound healing process engages inflammatory mediators, blood cells, extracellular matrix, and cell parenchyma, progressing through phases of hemostasis and inflammation, proliferation, and maturation and remodeling. Various factors, such as inadequate nutrition, hypoxia, immunosuppression, chronic illnesses, and post-surgical conditions, may impede the efficient healing of wounds ((Ulviani *et al*., 2017) [11].

Conclusion

Essential Oil Salve from Red Betel Leaf extract (Piper crocatum Linn.) effectively healed cut wounds in white rats. With bioactive compounds such as alkaloids, flavonoids, saponins, and tannins, the Saleb reached optimality at 9% concentration, approaching the healing ability of Bioplacenton® with the highest healing rate reaching 90% on day 14. This study offers a competitive alternative to conventional products in wound healing.

Research development is recommended to focus on exploring the mechanism of action of Essential Oil Saleb, further analysis of bioactive compounds, and toxicity testing at clinical doses. Comparative studies with similar products will provide a clearer picture of the potential and effectiveness of this Saleb in the market. Possible human clinical trials could be a further step to confirm the results of this study and support the clinical application of Essential Oil Saleb from Red Betel Leaf extract.

References

1. Abdullah Yeni, *et al*. Pengaruh Teknik Pengekstrakan Terhadap Kandungan Fitokimia Dan Sensoris Minuman Sirih Merah. *Jurnal Teknovasi: Jurnal Teknik Dan Inovasi*, Teknovasi, 2015:2(2):8-14.
2. Amaliah A, Sobari E, Mukminah N. Rendemen Dan Karakteristik Fisik Ekstrak Oleoresin Daun Sirih Hijau (Piper betle L.) Dengan Pelarut Heksan. Industrial Research Workshop,2019:10(1):273–278.
3. Apritya D, Sigit M, Yunani R, Lestari F. Pemanfaatan Infusa Daun Sirih Merah (Piper Crocatum) Sebagai Anti-Obesitas Pada Mencit (Mus Musculus). *Vitek: Bidang Kedokteran Hewan*, 2020, 50–57. Https://Doi.Org/10.30742/Jv.V10i0.49
4. Beon AS, Leki KGB. Identifikasi Komponen Fitokimia dalam Ekstrak Daun Sirih Merah (Piper crocatum). *STIKes Citra Husada Mandiri Kupang*, 2017.
5. Damarini S, Eliana E, Mariati M. Efektivitas Sirih Merah dalam Perawatan Luka Perineum di Bidan Praktik Mandiri. Kesmas: National Public Health Journal,2013:8(1):39. https://doi.org/10.21109/kesmas.v8i1.340
6. Diniatik. Uji Aktivitas Antivirus Eksrak Etanol Daun Sirih Merah (Piper Crocatum Ruitz & Pav) Terhadap Virus Newcastle Disease (Nd) Dan Profil Kromatografi Lapis Tipisnya. Pharmacy,2011:08(01):51–70.
7. Muhammad A’tourrohman, Malia Ulfah. Etnobotany Study on the Utilization of Sirih Types (Famili: Piperaceae) in Kalijambe Village, Kecamatan Bener, Purworejo District. *Biocelebes*,2020:14(3):278–278. https://doi.org/10.22487/bioceb.v14i3.15239
8. Sentat T, Permatasari R. Uji Aktivitas Ekstrak Etanol Daun Alpukat (Persea Americana Mill.) Terhadap Penyembuhan Luka Bakar Pada Punggung. Jurnal Ilmiah Manuntung,2015:1(2):100–107.
9. Syahrinastiti TA, Djamal A, Irawati L. Perbedaan Daya Hambat Ekstrak Daun Sirih Hijau (Piper betle L.) dan Daun Sirih Merah (Piper crocatum Ruiz & Pav) terhadap Pertumbuhan Escherichia coli. *Jurnal Kesehatan Andalas*,2015:4(2):421–424. https://doi.org/10.25077/jka.v4i2.275
10. Tomagola N. Aktivitas Antioksidan Ekstrak Kayu Manis (Cinnamomum Burmanii) Untuk Mengatasi Ketengikan (Rancidity) Pada Minyak Goreng. Journal of Chemical Process Engineering,2017:1(2):7. https://doi.org/10.33537/jcpe.v1i2.75
11. Ulviani F, Yusriadi Y, Khaerati K. Pengaruh Gel Ekstrak Daun Sirih Merah (Piper Crocatum Ruiz & Pav) Terhadap Penyembuhan Luka Bakar Pada Kelinci (Oryctolagus Cuniculus). Jurnal Farmasi Galenika (Galenika Journal of Pharmacy) (e-Journal),2017:2(2):103–110. https://doi.org/10.22487/j24428744.2017.v2.i2.5977
12. Widayani A, Cahyono E. Isolasi dan Uji Antioksidan Minyak Atsiri Daun Sirih Merah (Piper crocatum Ruiz & Pav.) pada Minyak Goreng Curah. Indonesian Journal of Chemical Science,2018:7(3):214–220. http://journal.unnes.ac.id/sju/index.php/ijcs
13. Windono NP, dan T. Sirih Merah (Piper crocatum Ruiz & Pav.) Kajian Pustaka Aspek Botani, 1 07 - 115 Kandungan Kimia, dan Aktivitas Farmakologi. Media Pharinaceutica Lndonesiana,2017:1(2):107–115.
14. Zubier F, Bramono K, Widaty S, Nilasari H, Louisa M, Rosana Y. Efikasi sabun ekstrak sirih merah dalam mengurangi gejala keputihan fisiologis. Majalah Kedokteran Indonesia,2010:70(1):9–14.