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In Vivo Incision Wound Healing Studies using Ethanolic Cinnamon's (*Cinnamomum burmannii*) Leaves Extract in White Male Rats

(Studi Penyembuhan Luka Insisi secara *In Vivo* Menggunakan Ekstrak Daun Kayu Manis (*Cinnamomum burmannii*) pada Tikus Putih Jantan)

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Abstract: Cinnamon leaves are traditional plants that contain secondary metabolites such as flavonoids, tannins, saponins, alkaloids, and phenols. All of these compounds play an essential role in the wound healing process. This study aimed to determine the effects of cinnamon (*Cinnamomum burmannii*) on incision wound healing in male rats. The research method used a post-test only control group design approach that uses five treatment groups: positive control (Bioplacenton® Gel), negative control (Vaseline flavum), and ethanol extract of cinnamon leaves at concentrations of 10%, 20%, and 30%. The results showed that the ethanol extract of cinnamon leaves affected wound healing, as observed from the day of wound healing, the AUC length of the wound, and levels of hydroxyproline. The statistical test results showed a significant difference between the groups ($p<0.05$), where the best concentration was 30%, followed by 20% and 10%. Therefore, we concluded that the ethanolic extract of cinnamon leaves can heal wounds in male rats.

Keywords: Cinnamon leaves, hydroxyproline, rat, wound healing

Abstrak: Daun kayu manis merupakan salah satu tanaman tradisional yang memiliki kandungan metabolit sekunder seperti flavonoid, tanin, saponin, alkaloid, dan fenol. Semua senyawa tersebut memiliki peran penting dalam proses penyembuhan luka. Penelitian ini bertujuan untuk mengetahui pengaruh pemberian kayu manis (*Cinnamomum burmannii*) terhadap penyembuhan luka insisi pada tikus jantan. Metode penelitian menggunakan pendekatan *Post Only Control Group Design* yang menggunakan lima kelompok perlakuan yaitu kontrol positif (Bioplacenton Gel), kontrol negatif (Vaseline flavum) dan ekstrak etanol daun kayu manis dengan konsentrasi 10%, 20% dan 30%. Hasil menunjukkan bahwa ekstrak etanol daun kayu manis berpengaruh terhadap penyembuhan luka yang dilihat dari hari penyembuhan luka, panjang AUC luka dan kadar hidroksiprolin. Hasil uji statistik menunjukkan perbedaan yang signifikan antar kelompok perlakuan ($p<0,05$) dimana konsentrasi terbaik adalah 30% diikuti konsentrasi 20% dan 10%. Oleh karena itu dapat disimpulkan bahwa ekstrak etanol daun kayu manis dapat menyembuhkan luka pada tikus jantan.

Kata kunci: Daun kayu manis, hidroksiprolin, penyembuhan luka, tikus

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INTRODUCTION

SKIN is one of the outermost parts of the body, so that can cause frequent injuries. Wounds cause skin epithelial damage that can arise as a result of trauma. The wound healing process is generally a complex cellular mechanism that focuses on continuously restoring damaged tissue. The stages of wound healing are homeostasis, inflammation, proliferation, and remodeling[1].

The wound healing process does not only occur naturally in the body but also will lead to impaired inflammatory healing. Wounds that heal in a long time with a more muscular immune system will be more susceptible to the emergence of infectious diseases caused by microorganisms[2]. Drugs commonly used are topical antiseptics. However, its use has side effects that can cause allergies. Traditional medicine has minimal side effects compared to synthetic drugs. Thus, many people started using traditional plants to deal with complaints of minor ailments[3].

The content of secondary metabolites in plants flavonoids, alkaloids, tannins, steroids, terpenoids, and saponins accelerates the wound healing process[4,5]. The flavonoids and alkaloids compounds play a role in the homeostatic process through vasoconstriction of blood vessels, free radical scavengers, hydrolysis inhibitors, and anti-inflammatory[6–8]. In addition, tannins act as wound astringents, and saponins act as antibacterials. These compounds have an important role in the wound healing process[9–12].

The cinnamon plant (*Cinnamomum burmannii*) is a plant that is well known for traditional medicinal therapy. Previous studies have proven that this plant has many properties in treating various types of disease. Cinnamon leaves have been tested for their effects, including as an antioxidant[13], antibacterial[14], anti-inflammatory, and others[15]. Previous research has also stated that cinnamon leaves extract contains compound flavonoids, alkaloids, tannins, steroids, terpenoids, and saponins[16]. Based on this problems, the researchers are interested in testing in vivo incision wound healing using ethanolic cinnamon's (*Cinnamomum burmannii*) leaves extract in white male rats.

MATERIALS AND METHOD

MATERIALS. The Cinnamon leaves, bioplacenton (Kalbe Pharma), aqua dest, veet (Unilever), hydrochloric acid (HCl)(Merck), Eter (Merck), Mayer Reagent, Dragendorf Reagent, Mg Powder, Amyl Alcohol, $FeCl_3$ n-hexane (Merck), 70% Ethanol (Brataco), NaOH, Chloroform (Merck), hydroxyproline (Sigma Aldrich), Aluminium foil, and Cuttonbud.

Tools. The tools used in this study were glassware (Pyrex), containers, UV-Vis Spectrophotometry (Thermo), Rotary evaporator (IKA), animal cages, and scapel.

METHOD. Plant Determination. Determination of cinnamon plants was conducted in the laboratory of Herbarium Andalas University.

Preparation of Cinnamons (Cinnamomum burmannii) Leaves Extract. The Cinnamon leaves was dried indoor for seven days, cut into small pieces and powdered using a belnder. Seven hundred and fifty grams of the cinnamon leaves powder was extracted using 70% ethanol solvent maceration method. The filtrate was evaporated in a rotary evaporator to obtain a good yield.

Phytochemical Screening. Phytochemical screening of the extract cinnamon leave employed standard procedures to reveal the presence of chemical constituents such as alkaloids, flavonoids, tannins, terpenes, and saponins.

Wound Healing Activity Test Cinnamon (*Cinnamomum burmannii*) Leaves. The test animals were divided into five treatment groups, each consisting of five rats. The test creatures were shaved on the back and given veet cream to evacuate the hair on the back after 24 hours, and after that, the treatment was carried out by giving an incision wound on the back of 2 cm and a depth of 2 mm using a scalpel blade. The treatments of the vaseline flavum, and ethanol extract of cinnamon leaves with concentrations of 10%, 20%, and 30%. The preparation is applied as much as 0.2 grams and given two times a day for 21 days.

Determination of Hydroxyproline Content. Main Solution Preparation: 50 mg of hydroxyproline dissolved in 50 mL of aqua dest to obtain a concentration of 1000 ppm. Then dilute by making a 100 ppm primary solution. Pipette 0.9 mL of the solution add up to 10 mL of aquadest. The solution is mixed with 1 mL of 0.02 N CuSO4, 1 mL of 2.5 N NaOH, and 1 mL of 6% H_2O_2. Then do the stir and heat using an oven at a temperature of 80^0C for five minutes. After cooling, measure λ using a UV-Vis Spectrophotometer with 560 nm.

Standard Curve: Make different concentration variations 9, 18, 27, 4,5 and 54 ppm. Weigh from 100 ppm primary hydroxyproline solution as much as 0.9, 1.8, 2.7, 3.6, 4.5, and 5.4 mL; add aqua dest up to 10 mL in a volumetric flask. Then observe the absorbance using spectrophotometry to obtain a standard curve of hydroxyproline.

Hydroxyproline Levels in Test Animals: On day

21 after treatment, rat skin preparations were taken. Then dry in the oven at 60^{0}C for 12 hours. Hydrolysis using 6 N HCl for 24 hours in an oven at 110^{0}C, then neutralized to obtain pH 7 using NaOH. Perform absorbance measurements to get the concentration of hydroxyproline in the skin.

Statistical Analysis. The results of the study were analyzed using One-Way ANOVA SPSS Program with a significance was 95%.

RESULTS AND DISCUSSION

Plant Determination. Determination of cinnamon plants conducted in the laboratory of Herbarium Andalas University No. 160/K-ID/ANDA/III/202 showed that the plant used was the species *Cinnamomum burmanni* (Nees & T.Nees) Blume.

Phytochemical Screening. The cinnamon leaves extract contained alkaloids, flavonoids, saponins, tannins, steroids, and triterpenoids. This result is in line with the results of research by Astika et al. (2021)[17].

Wound Healing Activity Cinnamon (Cinnamomum burmannii) Leaves Extract. The Research Ethics Committee approved all procedures to be performed on the test animals of Medical Faculty Andalas University No. 352/UN.16.2-FK/2021. Healing days were observed macroscopically. The dry process of the wound is characterized by the appearance of a scab on the skin as a marker that wound healing is in progress. The statistical test results showed a significant difference between the treatment groups in the observation of wound healing days and the AUC (Area Under Curve) wound length ($p<0.05$).

Table 1. Mean days of wound healing and AUC length of incisions in rats.

Group	Mean Days of Wound Healing± SEM	AUC Length of incisions±SEM
Positive Control	11.4±0.2^a	15.282±0.364^a
Negative Control	17.4±0.2^d	24.304±0.218^d
Extract 10%	14.2±0.3^c	20.996±0.342^c
Extract 20%	13.8±0.3^b	20.164±0.419^c
Extract 30%	13.4±0.2^b	18.026±0.220^b

Note: Different lowercase superscripts shown significant differences.

Table 1 shows the study result. The best extract concentration is 30% concentration. Then followed by a concentration of 20% and 10%. However, it did not exceed the effectiveness of the positive control (bioplacenton).

The wound healing process is a complex interaction of cellular and biochemical actions responsible for regaining the strength of the injured tissue. Injured skin will experience stages of the wound healing process starting from inflammation, wound contraction, re-epithelization, remodeling, and tissue formation through angiogenesis. The positive control used in this study was the bioplacenton which contains active placenta extract and neomycin sulfate. Placenta extract acts as a 'biogenic stimulator", which plays an essential role in accelerating the process of cell regeneration and wound healing. In contrast, neomycin sulfate works as an antibiotic that can kill various germs[18,19].

Natural materials have enormous potential in the management and treatment of wounds. Indonesia, since ancient times, has used a large number of plants used for the treatment of wounds, which leads to the ease of obtaining raw materials at low prices. There are many phytopharmaceutical laboratories whose research concentrates on the content of active compounds in plants that can have medicinal value. The content includes alkaloids, flavonoids, tannins, steroids, terpenoids, saponins, and phenolics[20].

Cinnamon is a plant that contains secondary metabolites that play an essential role in the wound healing process. All these compounds work synergistically. Alkaloids and flavonoids inhibit the formation of prostaglandin (COX-1 and COX-2), lipoxygenases (LOX and 5-LOX), and other inflammatory mediators[21]. Saponins act as potent antibacterials[22], and tannins work by accelerating the proliferation process of monocytes so that they can affect the number of macrophages[23]. An increase in the number of macrophages will affect the area around the wound, increasing the secretion of growth factors[24,25].

Hydroxyproline content in the skin plays a role in tissue formation during a wound as a parameter index of collagen levels in the skin. The higher level of hydroxyproline level can be interpreted as an increase in collagen formation during wound healing. The first stage of measuring hydroxyproline levels is making

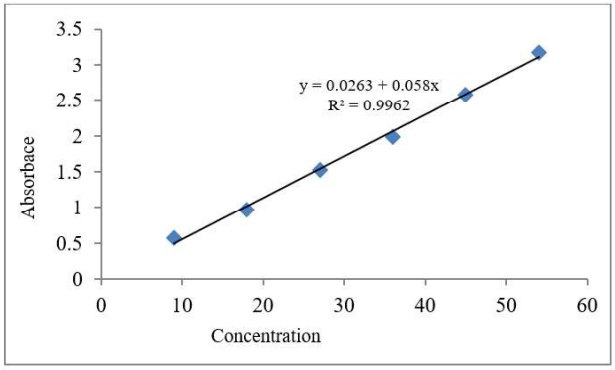

Figure 1. Hydroxyproline calibration curve.

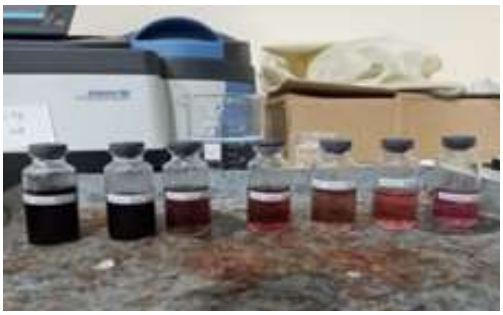

Figure 2. Rat skin hydroxyproline solution.

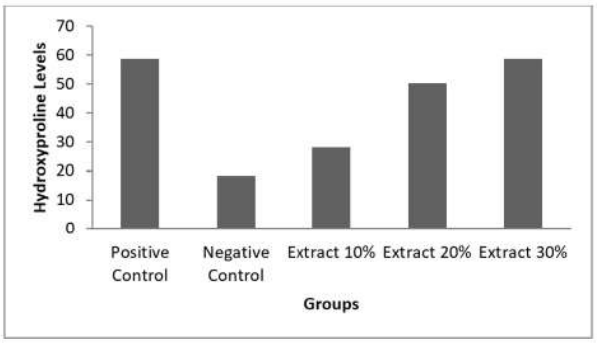

Figure 3. Hydroxyproline Levels.

a standard solution to obtain a standard curve used as linear regression data for determining hydroxyproline levels. The results obtained from this study are y = 0.0263 + 0.058x with a correlation coefficient value of r = 0.9962. Figure 1 shows the results.

The study results of hydroxyproline levels showed a significant difference for each treatment ($p<0,05$). The 30% ethanol extract administration gave the highest hydroxyproline value in the positive control. Figure 2 and Figure 3 show the result.

Collagen is the main protein of the extracellular matrix that contributes to wound healing[26,27]. The process of breaking down collagen will produce free hydroxyproline. Researchers can use a measurement of hydroxyproline levels as an index of collagen turnover. Besides this, results use a measurement of the hydroxyproline levels as an index of collagen turnover[28]. The results show a significant difference in hydroxyproline levels for the ethanol extract of cinnamon leaves and indicate an increase in breaking down granulation tissue from collagen maturation in injured skin[29,30].

CONCLUSION

Cinnamon leaves ethanol extract (10-30%) can be developed as an alternative for wound healing with the best concentration of 30%. Statistically showed a significant difference ($p<0.05$).

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REFERENCES

1. Haque SD, Al Amin AMM, Islam B, Nazneen N, Karim SN, Rahman MA. Antibacterial effect of Dimethyl sulfoxide extract of Aloe vera (Aloe *barbadensis*) leaf gel against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumoniae*. Mediscope. 2020 ;7(2):67-74.
2. Larouche J, Sheoran S, Maruyama K, Martino MM. Immune regulation of skin wound healing: mechanisms and novel therapeutic targets. Advances in wound care. 2018;7(7):209-31.
3. Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. Molecules. 2016;21(5):559.
4. Juneja K, Mishra R, Chauhan S, Gupta S, Roy P, Sircar D. Metabolite profiling and wound-healing activity of *Boerhavia diffusa* leaf extracts using in vitro and in vivo models. Journal of traditional and Complementary Medicine. 2020;10(1):52-9.
5. Moeini A, Pedram P, Makvandi P, Malinconico M, d'Ayala GG. Wound healing and antimicrobial effect of active secondary metabolites in chitosan-based wound dressings: A review. Carbohydrate polymers. 2020;233:115839.
6. Bapat UC, Mhapsekar DR. Phytochemical investigations and antimicrobial and anticancer activities of *Homonoia riparia* lour. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;6(11):237–43.
7. Aslam MS, Ahmad MS, Riaz H, Raza SA, Hussain S, Qureshi OS, Maria P, Hamzah Z, Javed O. Role of flavonoids as wound healing agent. Phytochemicals-source of antioxidants and role in disease prevention. 2018:95-102.
8. Carvalho MT, Araujo-Filho HG, Barreto AS, Quintans-Junior LJ, Quintans JS, Barreto RS. Wound healing properties of flavonoids: A systematic review highlighting the mechanisms of action. Phytomedicine. 2021;90:153636..
9. Su X, Liu X, Wang S, Li B, Pan T, Liu D, Wang F, Diao Y, Li K. Wound-healing promoting effect of total tannins from *Entada phaseoloides* (L.) Merr. in rats. Burns. 2017;43(4):830-8.
10. Ambreen M, Mirza SA. Evaluation of anti-inflammatory and wound healing potential of tannins isolated from leaf callus cultures of A*chyranthes aspera* and *Ocimum basilicum.* Pakistan Journal of Pharmaceutical Sciences. 2020;33(1):361-9.

11. Khan MI, Ahhmed A, Shin JH, Baek JS, Kim MY, Kim JD. Green tea seed isolated saponins exerts antibacterial effects against various strains of gram positive and gram negative bacteria, a comprehensive study in vitro and in vivo. Evidence-Based Complementary and Alternative Medicine. 2018;Jan:1-12.
12. Men SY, Huo QL, Shi L, Yan Y, Yang CC, Yu W, Liu BQ. *Panax notoginseng* saponins promotes cutaneous wound healing and suppresses scar formation in mice. Journal of Cosmetic Dermatology. 2020;19(2):529-34.
13. Latief M, Tafzi F, Saputra A. Aktivitas antioksidan ekstrak metanol beberapa bagian tanaman kayu manis (*Cinnamomum burmani*) asal Kabupaten Kerinci Provinsi Jambi. Prosiding Semirata FMIPA Universitas Lampung. 2013;233–6.
14. Vasconcelos NG, Croda J, Simionatto S. Antibacterial mechanisms of cinnamon and its constituents: A review. Microbial pathogenesis. 2018;120:198-203.
15. Błaszczyk N, Rosiak A, Kałużna-Czaplińska J. The potential role of cinnamon in human health. Forests. 2021;12(5):648.
16. Safratilofa S. Uji daya hambat ekstrak daun kayu manis (*Cinnamomum burmanii*) terhadap bakteri *Aeromonas hydrophila*. Jurnal Ilmiah Universitas Batanghari Jambi. 2017;16(1):98-103.
17. Astika, R.Y., Sani K. F., Elisma. Uji aktivitas antiinflamasi ekstrak etanol daun kayu manis (*Cinnamomum burmannii*) pada mencit putih jantan. Jurnal Ilmiah Manuntung. 2021;8(1):105–12.
18. Daisa F, Andrie M, Taurina W. The effectiveness test of oil phase ointment containing snakehead fish (*Channa striata*) extract on open stage ii acute wounded wistar strain male rats. Majalah Obat Tradisional. 2017;22(2):97-102.
19. 19. Sukmawan YP, Alifiar I, Nurdianti L, Ningsih WR. Wound healing effectivity of the ethanolic extracts of *Ageratum conyzoides* l. leaf (white and purple flower type) and *Centella asiatica* and astaxanthin combination gel preparation in animal model. Turkish Journal of Pharmaceutical Sciences. 2021;18(5):609:15.
20. Barreto RSS, Albuquerque-Júnior RLC, Araújo AAS, Almeida JRGS, Santos MRV, Barreto AS, et al. A systematic review of the wound-healing effects of monoterpenes and iridoid derivatives. Molecules. 2014;19(1):846–62.
21. Masfria, Hap UH, Nasution MP, Ilyas S. Cytotoxic activity, proliferation inhibition and apoptosis induction of *Rhaphidophora pinnata* (L.F.) schott chloroform fraction to MCF-7 cell line. International Journal of PharmTech Research. 2014; 6(4):1327-33,
22. Tagousop CN, Tamokou JD, Kengne IC, Ngnokam D, Voutquenne-Nazabadioko L. Antimicrobial activities of saponins from *Melanthera elliptica* and their synergistic effects with antibiotics against pathogenic phenotypes. Chemistry Central Journal. 2018;12(1):1-9.
23. Umami R, Malika R. Antibacterial test of binahong leaf extract ointment (*Anredera cordifolia*) against *Staphylococcus aureus* bacteria from diabetic wounds. PENBIOS: Jurnal Pendidikan Biologi dan Sains. 2020;5(01):1-7.
24. Kim SY, Nair MG. Macrophages in wound healing: activation and plasticity. Immunology and cell biology. 2019;97(3):258-67.
25. Krzyszczyk P, Schloss R, Palmer A, Berthiaume F. The role of macrophages in acute and chronic wound healing and interventions to promote pro-wound healing phenotypes. Frontiers in physiology. 2018;9:419:1-22.
26. Muralidhar A, Babu KS, Sankar TR, Reddanna P, Latha J. Wound healing activity of flavonoid fraction isolated from the stem bark of *Butea monosperma* (Lam) in albino wistar rats. European Journal of Experimental Biology. 2013;3(6):1-6.
27. Rahman N, Rahman H, Haris M, Mahmood R. Wound healing potentials of *Thevetia peruvian*a: antioxidants and inflammatory markers criteria. Journal of Traditional and Complementary Medicine. 2017;7(4):519-25.
28. Hendriati L, Hamid IS, Widodo T, Wandasari C, Risata PM. Effect of egg white gel againts burn healing on white rat (*Rattus novergicus*). Jurnal Ilmu Kefarmasian Indonesia. 2018;16(2):231-7.
29. Davoodi-Roodbordeii F, Afshar M, Haji Abas Tabrizi F, Choopani S, Torkaman G, Moayer F, Salimi M. Topical hydrogel containing *Fumaria vaillantii* Loisel. extract enhances wound healing in rats. BMC Complementary and Alternative Medicine. 2019;19(1):1-9.
30. Laksmitawati DR, Noor SU, Sumiyati Y, Hartanto A, Widowati W, Pratami DK. The effect of mesenchymal stem cell-conditioned medium gel on burn wound healing in rat, Veterinary World. 2022;15(4): 841-47.