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EFFECT OF CINNAMON LEAVES (*Cinnamomum burmannii*) EXTRACT GEL ON HYDROXYPROLINE LEVELS IN WOUND HEALING

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ABSTRACT

Wound often occur in daily activities. Cinnamon leaves are widely known to have pharmacological activity. The content of secondary metabolites in cinnamon leaves (Cinnamomum burmanii) is a support for development into pharmaceutical products in wound healing. Hydroxyproline is the main component of collagen, which plays a role in the healing process of cut wounds. The aims of this study was to determine the effect of gel preparation on hydroxydiproline levels in cases of Incisions. The research method is an experimental laboratory with a post test only control group design. The test animals used were 5 rabbits with 5 treatment groups, namely positive control (Bioplacenton®), negative control (Gel Base), Formula 1 (Gel Extract 2.5%), Formula 2 (Gel Extract 5%), and Formula 3 (Gel Extract 10%). The preparation was given topically on the back of the rabbit. Observations of hydroxyproline levels were carried out after 14 days of treatment. The results showed that there was a significant difference between the treatment groups ($p<0.05$) where the best formula was formula 2 with hydroxyproline levels of 44.13µg/mL which had an effectiveness close to positive control. Then followed by formula 3 and formula 1.

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INTRODUCTION

Wounds are conditions of damage or loss of body tissues caused by disruption of the body's protection system such as trauma to sharp or blunt objects, temperature changes, chemical substances, explosions, electric shocks or animal bites (Dong & Guo, 2021). The wound healing process are require complex process starting from the stages of homeostatic, inflammatory, proliferation and tissue remodeling. Incisions can occur due to intentional (surgical injuries) or accidental (extraidental wounds) due to sharp objects (Sani K et al., 2022).

Traditional medicinal plants are an alternative that is often used by the community to treat wounds. Previous study had mentioned that cinnamon leaves extract had an effect in wounds healing, both incisions and burns (Shi et al., 2021). After phytochemical screening, cinnamon leaves extract are contain compounds of secondary metabolites of alakaloids, flavonoids, saponins and tannins (Astika, et al., 2021). Where the compound acts as an anti-inflammatory and antibacterial for wound healing process can take place quickly.

Hydroxyproline levels are one of the proteins marking the collagen repair process in the skin. So that by determining the level of hydroxyproline in the skin that has experienced incisions, we will be able to find out the healing of the wound through the constituent components of collagen on the skin (Özbilgin et al., 2019). Topical administration of gel preparations is expected to provide an optimizing effect on wound healing because it directly acts on the wound site. Gel is a preparation whose easy manufacturing process, because it can to provide a cold feeling to the skin (Bharadwaj et al., 2019).

Based on the problems, researchers are interested in testing the effect of cinnamon leaves extract gel in wound healing in terms of hydroxyproline levels.

METHODS

Tools and Materials

Tool

Test Animal Cages, Surgical Blade No.11 (GEA), 1 mL syringe (Onemed), Animal Surgical Instruments, digital scales (Fulgid), measuring flask (Pyrex), erlenmenyer (Pyrex), *rotary evaporator* (IKA), beaker glass (Pyrex), uv-vis spectrophotometer (Shimizu).

Materials

Fresh Cinnamon Leaves were taken in the jambi city area, jambi province in July 2021. Aquadest, mayer reagent, dragendorft reagent, bioplacenton® (PT. Kalbe Farma), Karbopol (PT. Brataco), Glycerin (PT. Brataco), Propylene Glycol (PT. Brataco), Triethanolamine (PT. Brataco) Methyl Paraben (PT. Brataco), Propyl Paraben (PT. Brataco), Ethanol (PT. Brataco), Veet (PT. Reckitt Benckiser Indonesia), FeCl3 10% (Merck), Concentrated HCl (Merck), CuSO4 (Merck), NaOH (Merck), H2O2 30%, H2SO4 (Merck), 4-dimethylaminobenzaldehyde (Merck), NH4Cl (Merck).

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Cinnamon Leaves Extraction

Extraction of Cinnamon leaves (*Cinnamomun burmannii)* was performed using the maceration method using a 70% ethanol solvent. The simplicia powder was put into the vessel, then add a 70% ethanol solvent in a ratio of 1:10 until the powder was completely submerged, then covered and left for five days protected from light while stirring. Then the mixture was sembled and the pulp was reassessed with a 70% ethanol coating until submerged and left for 2 days, then poured so that a maserate was obtained. Then Maserat would concentrated with the help of a *rotary evaporator* at a temperature of no more than 40^{o}C and a viscous extract was obtained. The viscous extract obtained was then stored in the refrigerator in the *Rotary Evaporator* section and the extract obtained was calculated the results of the yield and a phytochemical screening examination was carried out.

Gel Formulations of Cinnamon Leaves Extract

Table 1. Gel Formulations of Cinnamon Leaves Extract

Material	Concentration (%)				Information
	K(-)	F1	F2	F3	
Cinnamon leaves ethanol extract	0	2.5	5	10	Active Substances
Carbopol 940	1.5	1.5	1.5	1.5	Gel base
Methyl parabens	0.18	0.18	0.18	0.18	Preservatives
Propyl parabens	0.02	0.02	0.02	0.02	Preservatives
Triethanolamine	1	1	1	1	Pendapar
Glycerine	5	5	5	5	Humectants
Propilenglikol	10	10	10	10	Enhancher
Aquadest	ad 100	ad 100	ad 100	ad 100	Solvent

Notes :

F0= Gel base that does not contain cinnamon leaves ethanol extract

F1 = Gel base containing 2.5% cinnamon leaves ethanol extract

F2 = Gel base containing 5% cinnamon leaves ethanol extract

F3 = Gel base containing 10% cinnamon leaves ethanol extract

Carbopol was developed with aquadest in mortar until it expands. Methyl paraben dissolved in glycerin stir until dissolved in beaker glass. In different mortars Cinnamon leaves extract with various concentrations was grinded until the texture becomes soft then add propilenglikol scour to form a homogeneous preparation. The expanded carbopole was

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grinded then add TEA bit by bit until it forms a gel. A mixture of glycerin, methylparaben and active substances was mixed in the stinger to form a gel. Add the rest of the aquadest until homogeny. Then the gel was carried out an evaluation of the preparation. Then the gel that had formed was attached as much as 0.2 grams to the hipafix plaster. Each animal used in this study was 5 rats per group.

Wound Healing Activity

The day before the wound was made, the test animals were shaved on the back area Which had previously been cleaned with 70% alcohol. Next, a 2 cm long incision was made on the back by holding the rabbit's skin with tweezers. Then a wound was made using a sterilized *cutter*. This experiment were used Positive Control (Bioplacenton®), Formula 0 (Gel Base), Formula 1 (Cinnamon leaves extract concentration 2.5%), Formula 2 (Cinnamon leaves extract concentration 5%), and Formula 3 (Cinnamon leaves extract concentration 10%).

Determination of Maximum Wavelength

Prepare the main solution of hydroxyproline by weighing as much as 125 mg of standard hydroxyproline powder and then put it into a 250 mL volumetric flask and dissolved with aquabidest. Then take the main solution of hydroxyproline pipette as much as 10 mL, put it into a 100 mL volumetric flask, add aquabidest to the mark. Then a 0.9mL pipette was added to a 10 mL volumetric flask and added 1 mL of 0.01N $CuSO_4$, 1 mL of 2.5 N NaOH, and 1 mL of 6% H_2O_2. The solution was stirred and incubated at 80^0C for 5 minutes. After the solution was cooled and added 4 mL of 3 M H_2SO_4 and 2 mL of 2-dimethylaminobenzaldehyde 5%, incubated again at 70^0C for 16 minutes, cooled at 20^0C and measured absorption using UV-Vis spectrophotometry at a wavelength of 200-800 nm and determined the length. maximum wave.

Manufacture of Hydroxyproline Standard Curves

The 50 ppm solution were made 6 variations of different solution concentrations in 10mL volumetric flasks with variations of 9, 18, 27, 36, 45, and 54 ppm which were made by taking 0.9, 1.8, 2.7, 3.6, 4.5 and 5.4 mL which were inserted in a 10 mL volumetric flask until the limit mark. Pipettes of 1 mL of hydroxyproline solution in each concentration variation were mixed as much as 1 mL $CuSO_4$ 0.01N, add 1 mL NaOH 2.5 N, add1 mL H_2O_2 6%. Then do stirring and heat at a temperature of 80^0C for 5 minutes. Then cool add 4 mL H2SO4 3 M and 2 mL 2-dimethylamiinobenzaldehyde 5% heat at a temperature of 70^0C for 16 minutes cooled and measured absorption using UV-Vis spectrophotometry at a wavelength of 560nm. For blanks use aquadest with the same treatment as the standard curve.

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Determination of Hydroxyproline Levels

On the skin of the wound in the biopsy was carried out on the 15th day. The skin tissue was dried in an oven at a temperature of 60 ^{0}C for 12 hours and hydrolyzed with HCl 6N for 24 hours in an oven with a temperature of 110 ^{0}C. then was carried out neutralized to pH 7 using an NH_4Cl 0.2 M buffer with its base NH_4OH 0.2M and NaOH 2.5N determination of hydroxyproline content was treated the same as the standard solution. The amount of hydroxyproline was determined based on the results of the standard curve.

Data Analysis

Analysis of research data were carried out in two ways, namely descriptively (extract characteristics) and using the SPSS Program 23 one-way anova test (Hydroxyproline Levels) with a confidence level of 95%.

RESULTS AND DISCUSSION

Plant Determination

This study was used part of the cinnamon plant, namely fresh dark green cinnamon leaves has obtained from Pungut Hilir Village, East Air Hangat District, Kerinci Regency. Before the study was carried out, the sample was determinated to ascertain the correctness and specifications. Samples were determinated at the "Herbarium Jatinangor Laboratory of Plant Taxonomy" Department of Biology FMIPA of Padjajaran University. The results of the sample identification with No.22/HB/01/2022, was showed that the sample came from the family *Lauraceae* and the species *Cinnamomum burmannii* (Ness & T.Ness) Blume.

Cinnamon Leaves Ethanol Extract

Cinnamon leaves were extracted using the maceration method. A total of 750 grams of cinnamon leaves powder was extracted by the maceration method for 1 day and remaseration for 2 days. The result of the extraction process was obtained as much as 6 liters of maserate or filtrate, then filtered and concentrated using a *Rotary evaporator* at a temperature of 50 ° C until 103 grams of thick extract with an yield of 13.73% was obtained. The principle of this tool is to remove the solvent contained in the maserat by evaporating until only the extraction compound is left called a viscous extract.

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Phytochemical Screening

Table 2. Phytochemical Screening Test of Cinnamon Leaves Ethanol Extract

Phytochemical Test	Observations
Flavonoids	+
Alkaloids	+
Saponins	+
Tannins	+
Terpenoids	+
Phenol	+

Note, (+) : shows a positive result

Based on the table above, it can be known that cinnamon leaves contain secondary metabolites in the form of flavonoids, alkaloids, tannins and saponins. This is in accordance with research that has been carried out by Nursofia et al., (2021) states that cinnamon leaves were contain alkaloid compounds, saponins, flavonoids, phenolics, steroids and tannins.

Determination of Hydroxyproline Levels

The negative control used in this study was the gel base which is a carrier of the active substance in the dosage formulation. While the positive control used was Bioplacenton. Bioplastentone is a gel-shaped pharmaceutical preparation that contains placenta extract to function as a biogenic stimulator to accelerate cell regeneration. Meanwhile, neomycin sulphate acts as an antibiotic to kill bacteria with the power to prevent the process of infection or the formation of pus in the wound (Sani K et al., 2022).

Determination of hydroxyproline levels was carried out on the 15th day, which was the day when the proliferation phase takes place. Proliferation is a phase that lasts for the first 48 hours (2 days) after the occurrence of the wound until the 15th day. In this phase, the undifferentiated fibroblast cells begin collagen synthesis which will replace the connective tissue in the wound area to build new tissue or scar tissue that causes contraction of the fibers, so that the wounds were narrowed. The content of hydroxyproline in the cut-scar skin tissue was measured using a UV-Vis spectrophotometer and using a standard hydroxyproline curve by looking for linearity, where linearity is used to calculate hydroxyproline levels.

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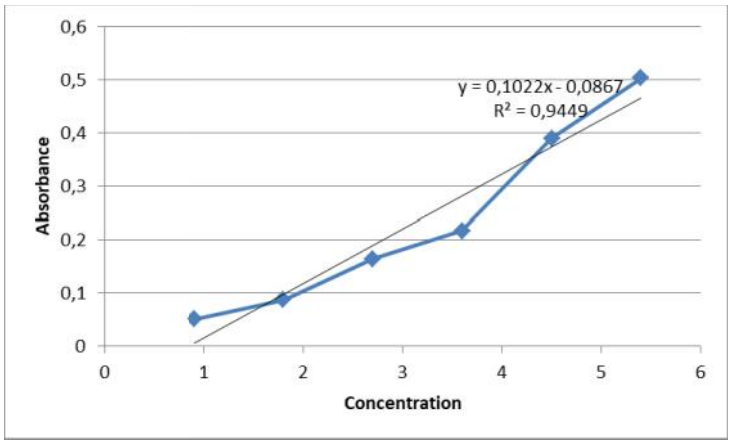

Figure 1. Standard Curve of Hydroxipoline Graph

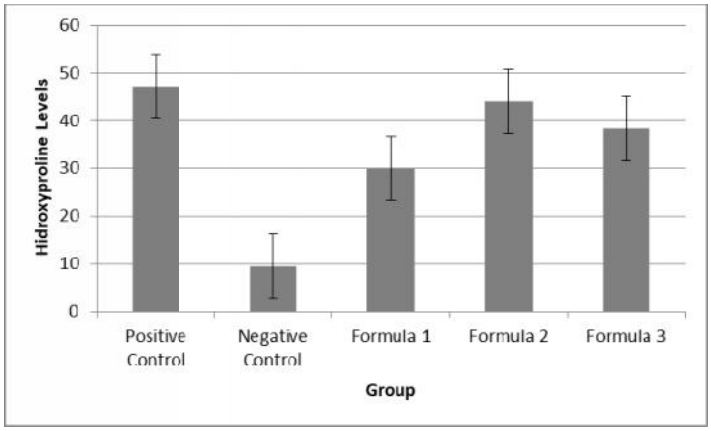

Figure 2. Hydroxypoline Levels of Skin Tissue

The results of the statistical test were showed a significant difference between the treatment groups ($p<0.05$). Duncan's further tests were showed that formula 2 provides the best effect in wound healing. This can be seen from the level of skin hydroxyproline obtained in each treatment. Then followed by formula 3 and formula 1. This problem was occur because more and more extracts will trigger the appearance of histamine when experiencing wounds related to wound inflammation. In certain conditions, mast cells if given high concentrations of drugs will increase the permeability of blood vessels to plasma fluid and cause inflammatory processes (Larouche et al., 2018).

The content of secondary metabolites in cinnamon leaves extract that play a role in wound healing are alkaloids, flavonoids, saponins, terpenoids and tannins. Alkaloids and flavonoids have a role as anti-inflammatories and microorganisms through inhibition of the cyclooxygenase enzyme and preventing the biosynthesis of the formation of prostaglandins

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and leukottrienes so that they can provide an effect of reducing the number of leukocytes accumulated on the wounded area will become injured (Carvalho et al., 2021). Saponins and terpenoids were act as antibacterials and anti-inflammatories by inhibiting the release of pro-inflammatory substances such as INOS, IL, and TNF-α so that there will be a decrease in exudate fluid and inhibit the permeability of the vascular system and d disrupting the function of bacterial cell membranes(Ibrahim et al., 2018; Men et al., 2020). Finally, tannins have a function as an astringet that can deposit proteins on the surface of cells with low permeability so as to help the process of closing skin pores, hardening the skin, reducing exudate and light bleeding (Ambreen & Mirza, 2020).

CONCLUSION

Cinnamon leaves extract gel has an effect as a healer of incisions wounds in the review of hydroxyproline analysis which statistically has a meaningful difference ($p<0.05$). Formula 2 with an extract concentration of 5% is the best formula in terms of hydroxyproline levels. The evaluation value for 2 weeks of observation was 44.13±0.33 µg/mL.

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