Hydrogel Activity Test from Kirinyu Leaf Ethanol Extract (Chromolaena odorata (L.) R. King & H. Rob) and Combination of Kirinyu Leaf Ethanol Extract with Collagen as a Healing Wound Excision.

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A B S T R A C T

Objective: The purpose of this study was to determine the activity of the kirinyu leaf ethanol extract hydrogel preparation and the combination of the kirinyu leaf ethanol extract with collagen as a healing wound excision.

Method: This research is experimental, where the ethanol extracts of Kirinyu leaves and collagen are formulated in the form of hydrogel preparations and tested on the healing of excision wounds in rats. Testing wound healing activities were divided into 6 groups. The Group 1 (negative control) were given hydrogel base without Kirinyu leaf ethanol extract in combination with collagen, group 2 (positive control) were given Octenilin®/gel, groups 3 and 4, were given hydrogel of Kirinyu leaf ethanol extract while groups 5 and 6 were given hydrogel extract of kirinyu leaves combined with collagen.

Result: The results of percent reduction in wound diameter showed that groups 3 and 4 gave significant differences compared to group 1 which began to be seen on the 10th day (p <0.05). While groups 5 and 6 gave significant differences that began to be seen on the 8th day (p<0.05).

Conclusion: Hydrogel preparation in group 6 has the most potential in healing excision wounds.

Keywords: Kirinyu leaf, collagen, hydrogel, excision wound.

ARTICLE INFO: Received 25 Nov 2019; Review Completed 13 Jan 2020; Accepted 25 Jan 2020; Available online 15 Feb. 2020

Cite this article as:
Thursina C. S.*, Reveny J, Sumaiyah S. Hydrogel Activity Test from Kirinyu Leaf Ethanol Extract (Chromolaena odorata (L) R. King & H. Rob) and Combination of Kirinyu Leaf Ethanol Extract with Collagen As A Healing Wound Excision, Asian Journal of Pharmaceutical Research and Development. 2020; 8(1):35-37.
DOI: http://dx.doi.org/10.22270/ajprd.v8i1.651

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INTRODUCTION

Injury is a disorder or damage from the integrity of the skin. One type of wound is an excision wound, which is the loss of skin as a whole and is widespread, causing a large amount of tissue to be lost 1. Kirinyu leaves contain several main compounds such as tannins, phenols, flavonoids, saponins and steroids. Tannins and flavonoids are one of the compounds contained in the leaves of Kirinyu which can accelerate the process of wound healing due to its efficacy as an astringent, which has a role in shrinking wounds and increasing the rate of epithelialization. Flavonoids and saponins also have the ability as a cleanser and antiseptic that functions to kill or prevent the growth of microorganisms that commonly occur in wounds so that the wound does not experience severe infections 2-3. Collagen is a fibrous protein that gives strength and flexibility to tissues and bones and plays an important role for other tissues, including the skin and tendons. This compound is the main protein that makes up the extracellular matrix component 4.

Hydrogels are semisolid preparations formed by hydrophilic tissue. Hydrogel is ideally used as a wound cover material because it can help remove dead tissue. Hydrogels are able to absorb water or biological fluids so that the wound area becomes dry and is able to provide a cold feeling to the skin caused by the slow evaporation of water so as to reduce swelling around the wound so that it will speed up the wound healing process 5-6.
Preparation of Extracts
Making extracts is done by maceration. 500 g of simplicia powder soaked with 70% alcohol for 6 hours while stirring occasionally. Then let stand for 18 hours. Separate maserat by filtering. Repeat the search process twice with the same type and amount of solvent. Furthermore, all maserates are collected, then evaporated with a rotary evaporator at ± 50°C until a thick extract is obtained.

Formulation of Hydrogel
The process of making hydrogel begins with the development of carbopol and Na-CMC in 20 ml distilled water. then stir until homogeneous. Add glycerin and propylene glycol and complete triethanolamine while stirring constantly and add distilled water. Then add thick extract with various concentrations that have been dissolved into 2 ml of distilled water to produce hydrogel ethanolic extract of Kirinyu leaf and add ethanol extract of Kirinyu leaf and collagen powder which has been dissolved into 1 ml of 0.5 M acetic acid to produce hydrogel ethanol extract Kirinyu leaf combination with collagen. The percentage of the ingredients of hydrogel formula (Table 1) was modified from the hydrogel formula which was carried out in the previous study by Edy, et al.6

Table 1: Hydrogel formulation of kirinyu leaf ethanol extract and combination of kirinyu leaf ethanol extract with collagen.

| Composition of Hydrogel | Formulation (%) |
|-------------------------|-----------------|
|                         | F0   | F1   | F2   | F1K | F2K |
| Extract                 | -    | 3    | 5    | 3   | 5   |
| Collagen                | -    | -    | -    | -   | -   |
| Carbopol 940            | 1    | 1    | 1    | 1   | 1   |
| Na-CMC                  | 1    | 1    | 1    | 1   | 1   |
| Propylene glycol        | 2    | 2    | 2    | 2   | 2   |
| Triethanolamine         | 1    | 1    | 1    | 1   | 1   |
| Glycerin                | 12.5 | 12.5 | 12.5 | 12.5| 12.5 |
| Distilled Water ad      | 100  | 100  | 100  | 100 | 100 |

Physical Evaluation of Hydrogel
Physical evaluation of the preparation includes organoleptic, homogeneity, pH measurement and viscosity of the preparation. Stability test is carried out for 90 days.

Experimental Animal
The experimental animals used in this study were male Wistar strain rats (Rattus Norvegicus) weighing 200-250 g. The experimental protocol was approved by the Institutional Animal Ethics Committee.

Wound Making for Test Animals
The rats were acclimatized for 7 days, then anesthetized using ketamine HCl, then the hair was shaved on the back of the rat to be made wound then cleaned with cotton that was given 70% alcohol. The wound pattern is made in a circle with a 20 mm diameter. Then made a wound with the size of a sign that has been made in the form of a circle with a 20 mm diameter. Then made a wound with a characteristic smell of Kirinyu leaves.

Table 2: The results of pH measurements of hydrogel

| Formulation | pH     |
|-------------|--------|
|             | 0 Days | 7 Days | 14 Days | 21 Days | 28 Days | 90 Days |
| F0          | 6.3    | 6.3    | 6.3     | 6.3     | 6.3     | 6.3     |
| F1          | 6.3    | 6.3    | 6.3     | 6.3     | 6.2     | 5.9     |
| F2          | 6.1    | 6.1    | 6.1     | 6.1     | 6.0     | 5.4     |
| F1K         | 5.8    | 5.8    | 5.8     | 5.8     | 5.7     | 5.3     |
| F2K         | 5.7    | 5.7    | 5.7     | 5.7     | 5.5     | 4.8     |
Viscosity test

The results of determining the hydrogel viscosity were carried out using a NDJ-8S viscometer on all preparations. Viscosity testing aims to determine the viscosity value of a substance. Good viscosity of hydrogel preparation is 50-150 dPa.s. Table 3 shows the results of the viscosity values of the four formulas for 90 days at room temperature.

| Formulation | Viscosity (dPa.s) |
|-------------|-----------------|
|             | 0 Days | 7 Days | 14 Days | 21 Days | 28 Days | 90 Days |
| F0          | 99,9   | 99,9   | 99,9    | 99,9    | 99,9    | 99,9    |
| F1          | 99,9   | 99,9   | 99,9    | 99,9    | 99,9    | 99,9    |
| F2          | 99,9   | 99,9   | 99,9    | 99,9    | 95,1    | 70,8    |
| F1K         | 99,9   | 99,9   | 99,9    | 99,9    | 99,9    | 70,2    |
| F2K         | 99,9   | 99,9   | 99,9    | 99,9    | 96,4    | 85,3    | 63,4    |

Percentage of wound diameter reduction

Test results on excision wound testing were analyzed using the SPSS program 25, One Way ANOVA method. Group 1 shows the percentage of wound diameter shrinkage of 100% on the 28th day. Group 2 shows the percentage of wound diameter shrinkage of 100% on the 16th day. Groups 4, 5 and 6 show the percentage of wound diameter shrinkage of 100% on the 18th day. In group 3 the percentage diameter of 100% shrinkage on the 21st day on the new graph is seen on the 22nd day.

![Figure 1: Graph of percentage reduction in wound diameter in each group observed on the day of observation](image)

The results of statistical analysis showed that the group given hydrogel ethanol extract of kirinyu leaf gave a significant difference compared to the group given hydrogel without ethanol extract of kirinyu leaf which began to be seen on the 10th day (p < 0.05). The group that was given hydrogel ethanol extract of kirinyu leaf with collagen also gave a significant difference with the group that was given hydrogel without ethanol extract of kirinyu leaf which began to be seen on the 8th day (p < 0.05).

CONCLUSION

Hydrogel preparation of 5% kirinyu leaf ethanol extract with the addition of 1% collagen has the greatest potential in wound healing.

ACKNOWLEDGMENTS

This research was facilitated by the Faculty of the Pharmacy University of Sumatera Utara in 2019.

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