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THE EFFECTIVENESS OF ETHANOL EXTRACT BOTTO'-BOTTO' LEAVES (Chromolaena odorata L.) IN TRANSDERMAL PATCH FORMULATION AS MEDICATION IN WOUND HEALING

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ABSTRACT

Botto'-botto' plant (Chromolaena odorata L.) is a native Indonesian plant that belongs to the Asteraceae family and is known to have a high flavonoid content and antioxidant activity that can slow down the oxidation process to prevent infection while accelerating wound healing. botto'-botto' is designed as a patch to make it easier to use and provide the most effective treatment. The purpose of this study was to make a patch preparation and to investigate its effects on wound healing. The patch was formulated then tested for physical properties (organoleptic pH, humidity, thickness, weight uniformity, and folding endurance test) and their activity against wounds experimentally using rabbits as an animal test with 4 (four) treatment groups, namely the control group, botto'-botto patch 10%, 20%, and 30%. Wounds were made with an area of 2 cm and 0.1 cm deep. The wound was plastered with a patch and observed for 18 days. Data were analyzed statistically using ANOVA. The results showed that the patch formulation of leaves extract botto'-botto' affects for wound healing in rabbits significantly, with $p<0.05$. The patch formulation of ethanol extract botto'-botto' with a concentration of 30% had the best and fastest healing effect among all formulas.

Keywords *: Botto'-botto' leaves extract, wound healing, transdermal patch*

INTRODUCTION

The skin is one of the human organs that has a protective effect from external disturbances such as exposure to water, air, ultraviolet, temperature, and external stimulation. When the skin has problems, the work function of the skin will be disrupted. Damage to tissue or anatomical and physiological structures of the skin may be caused by physical contact with sharp or blunt objects, animal bites, results of medical procedures, heat sources, chemical substances, and others (Purnama *et al*, 2017). Damage to the skin requires a long healing process of at least 5 to 10 days (Velnar *et al*, 2009). During the healing period, these wounds can cause new problems such as infection due to the growth of fungi, bacteria, and parasites. To overcome this, routine external treatment is needed to use ointments, gels, and creams widely circulated in pharmacies.

Botto'-botto' leaves, from the Asteraceae family, contain many chemical compounds

that have antioxidant and antibacterial effects. This activity can inhibit the oxidation process to accelerate wound healing and prevent infection in the wound (Ngozi, *et al*, 2009).

Botto'-botto' leaves extract were packaged in modern preparations in the form of patches for easier use. A transdermal patch is a pharmaceutical product for external use that can stick to the skin, and consists of two layers, an adhesive polymer layer and an impermeable backing layer (Koyi, *et al*, 2012). The patch has a function as a drug delivery agent or active ingredient to enter and penetrate the layers of the skin and then work on the affected skin tissue. The patch preparation is quite effective because it can reduce the number of uses, maintain the consistency of the availability of the drug in the plasma stream, avoid first-pass metabolism during oral administration, for the case of patients who cannot swallow the drugs, and the use is easily discontinued if it occurs toxic effect.

MATERIAL AND METHODS

Materials and Instrument

Botto'-botto leaves were taken at Parangloe, Chitosan, HPMC, glicerin, and tween 80 were purchased in Citra Persada acetic acid, 70% ethanol, and aquadest. This study used extraction tools, oven (Memmert®), pH meter, patch mold.

Sixt *Oryctolagus cuniculus* of or known as laboratory rabbit, weighing 1.2 – 1.5 kg, were used in this study.

Extract Preparation

Botto'-botto' leaves powder (2 kg) were extracted by maceration using 2 L of ethanol 70% for 48 hours. Extraction were evaporated using an evaporator at 60°C 60-80 rpm.

Transdermal Patch Formulation

Chitosan polymer solution was prepared using 1% acetic acid (A), HPMC polymer was prepared with a mixture of distilled water: ethanol (8:2) (B). Solution A was mixed with solution B, homogenized using a magnetic stirrer at 350 rpm, then tween 80 was added. The extract is then added to the solution mixture and stirred for 30 minutes and then add glycerin, stirring to homogenize. Then it is printed with a patch mold and allowed to stand for 24 hours (Syakri, 2019). The patch preparations in this study used different extract concentrations of botto'botto' leaves extract and without botto'botto' leaves extract as a control (table 1), chitosan and HPMC as a

Table 1. Patch Formulation

Materials	Formulas			
	FI	FII	FIII	FIV
Botto'-botto' extract (mg)	-	50	100	150
Chitosan: HPMC	1:1	1:1	1:1	1:1
Gliserin (w/w)	20%	20%	20%	20%
Twen 80 (w/w)	3%	3%	3%	3%

matrix, glycerin as a plasticizer, and tween 80 as emulgator.

Preparation and Treatment of Animal Test

The acclimatization process lasts for seven days. Experimental animals were given standardized concentrate feed and drinking water ad libitum. The rabbit's back fur is shaved before the patch is applied.

Evaluation of Transdermal Patch

Organoleptic

The organoleptic test was carried out by observing the smell, color, and texture of the botto'-botto' leaves extract patch matrix.

Superficial pH

For each of the formulation, 1 ml of distilled water was added to the surface of each patch (12 determinations), after waiting 120 min, the pH of the surface was determined with a digital pH meter (Almazan *et al*, 2020).

Moisture loss

The percentage moisture loss was carried out to evaluate integrity of the film in dry conditions. Patch were weighed and kept in a desiccator containing anhydrous calcium chloride. After one days, the patches were taken out and reweighed (Priya, *et al*, 2011). The percentage moisture loss was calculated using the formula

$$\% \text{ Moisture absorption} = \frac{Final\ weight - Initial\ weight}{Initial\ weight} x\ 100$$

Thickness Test

The thickness of patches was assessed using a micrometer (Vernir caliper®). For each formulation, three randomly selected patches with surface area 1 cm^2 were used (Pandekal *et al*, 2012).

Weight Uniformity Test

The patches are dried at 60°C before weighing. The weight uniformity of the patch is measured by cutting andweighing the 1 cm^2 piece of five patches and then calculating the weight variation. The mean of the 5 is taken as the weight of the patch (Jhawat, *et al*, 2013).

Folding Endurance Test

Folding endurance of patches was determined manually by repeatedly folding a film at the same place for approximately 300 times or until it ruptured. The number of folding required to break or crack a patch was taken as the folding endurance (Jhawat *et al*, 2013; Pandekal *et al*, 2012).

Wound Healing Test

The test was divided into 4 group treatments. Group I, was a control group using a patch preparation without extract. Group II, III and IV were treated with a patch formulation containing 10%, 20%, and 30% botto'-botto' leaves extract, respectively. The observation was carried out by looking and measuring the length of wound healing using a calliper by observing the presence of erythema, swelling, and wound closure. The initial wound size is 2 cm with a depth of 0.1 cm. Observations were made for 18 days.

Data Analysis

Data were analyzed using a one-way ANOVA test. The results were obtained significantly if the $p < 0.05$.

RESULTS AND DISCUSSION

Botto'-botto' leaves extract

The extraction result has a brownish green color. The yield obtained from the maceration is 22%. Concentration choose based on previous research that concentration 10% of extract was good at wound healing in cream preparation then we used another concentration to test it in transdermal patch preparation (Handayani, 2018)

Organoleptic

Organoleptic tests were observed with the patch preparation's smell, taste, color, and consistency. In addition, the physical characteristics of the transdermal patch preparation were followed to ensure the alignment of the results of the visual patch condition. Result of the organoleptic botto'-botto' leaves extract patch can see in table 2.

Table 2. Organoleptic test of Botto'-botto' leaves extract patch.

Organoleptic	Formula			
	FI	FII	FII	FIV
Odor	Odorless	Odorless	odorless	Odorless
Color	Clear	Brownish	Brown	Brown
Texture	Elastic/ semi solid	Elastic/ semi solid	Elastic/ semi solid	Elastic/ semi solid

pH Test

The pH of the patches made was in the range of 5.56 - 6.05 (table 3) and in accordance with the pH of the skin. pH of patches that are too acidic or alkaline can irritate the skin (Kumar *et al*, 2008).

Moisture loss

The moisture loss aims to determine the moisture contents of the patch. This value is expressed in percent of the difference between the initial weight and the final weight of the patch after being stored in a desiccator containing two silica gels for 24 hours. According to Prajapati et.al, 2011, low water content indicates a perfect solvent evaporation process. In addition, the low water content can also keep the patch more stable, flexible, and not brittle. The proportion of humidity in each formula was obtained. The expected humidity content is less than 10% (Kumar *et al*, 2013).

A low water content percentage can prevent microbes contamination (Zakir, *et al*, 2015). The result (table 4) shows that only FIV has humidity less than 10% while FI, FII and FIIIare more than 10%, meaning that FIV has good moisture.

Table 3. pH of Botto'-botto' leaves extract patch.

Formulas	pH
FI	6,05 ± 0,44
FII	5,58 ± 0,25
FIII	5,86 ± 0,08
FIV	5,56 ± 0,081

Table 4. Humidity of Botto'-botto' leaves extract patch.

Formulas	Humidity (%)
FI	23,9
FII	16,4
FIII	14,9
FIV	8,4

Thickness

The thickness test of the patch is carried out to know the uniformity of the patch thickness. If the patch has the same thickness, it is assumed to have a uniform weight. Therefore, it can be said that the active substance contained is also consistent. Based on table 5, the average value and standard deviation (SD) obtained from the thickness test are as follows, namely for control formula 0.21 ± 0.029 mm, the formula I (FI) 0.22 ± 0.016 mm, formula II (FII) 0 .20 ± 0.013 mm and formula III (FIII) 0.21 ± 0.009 mm.

Table 5. Thickness of Botto'-botto' leaves extract patch.

Formulas	Thickness (mm)
FI	0,213 ± 0,03
FII	0,223 ± 0,02
FIII	0,205 ± 0,01
FIV	0,211 ± 0,01

Uniformity

The test was carried out to determine the uniformity of the patch. Uniform weight can be assumed as the uniformity of substance contained in the patch. Based on the test results (table 6), the average weight value and standard deviation (SD) for the control formula (FK) is 1.169 ± 505 mg, FI is 26.73 ± 483.5 mg, FII is 23, 23 ± 485 mg, and FIII is 0.516 ± 500 mg. Therefore, the smallest to largest weight uniformity is FI, FII, FIII and FK. According to the Indonesian Pharmacopoeia, a good weight uniformity parameter can be seen from the CV (Coefficient of Variation) value, which is less than or equal to 5%. The results of each formula obtained a CV value of less than 5%, meaning that the weight uniformity was good.

Table 6. Uniformity of Botto'-botto' leaves extract patch.

Formulas	Weight (mg)	Coefficient of variation (CV)
FI	505,17 ± 1,17	0,231
FII	483,5 ± 26,73	5,529
FIII	485,00 ±23,24	4,791
FIV	500,33 ± 0,52	0,103

Folding Endurance

A good patch must have strong and elastic properties. The integrity of the patch, when applied to the skin, is indicated by its good folding resistance, so it is hoped that the patch will not tear easily during the period of use. Patches have an excellent folding resistance

Table 7. Folding Endurance of Botto'-botto' leaves extract patch.

Formulas	Average (times)
FI	343
FII	340,8
FIII	151,3
FIV	415,6

value if they can be folded 290-300 times without tearing. Therefore, in this study, a folding endurance test was conducted to determine how often the patch can be folded until a tear appears. This test is done manually. One of the components in the patch formulation is a plasticizer and is a factor that can affect the elasticity and foldability of the patch preparation. Folding endurance of tobo'-tobo' leaves extract patch can see in table 7.

Wound Healing

Cuts (*Vulnus scissum*), are wounds characterized by the surface of the wound edges in the form of straight and regular lines (Orsted *et al*, 2010). The process of wound healing in the human body can take place naturally but this process requires a long term, usually ranging from 5 to 10 days (Velnar *et al*, 2009).

Botto'-botto' leaves contain many active compounds used in the healing process. Some of the main compounds such as tannin, phenolic, flavonoid, saponin and steroid compounds, essential oils, alpa-pinene, cadinene, campfer, limonene, beta-caryophyllene and candinol isomers, the compounds in this plant can help in the healing process, especially in the antioxidant group

Table 8. Wound Healing activity of Botto'-botto' leaves extract patch.

Day	Diameter of the incision (cm)			
	FI	FII	FIII	FIV
1	2	2	2	2
2	2	1,98	1,98	1,95
3	1,95	1,88	1,88	1.83
4	1,9	1,78	1,78	1,73
5	1,8	1,68	1,68	1,6
6	1,7	1,56	1,51	1,43
7	1,6	1,46	1,31	1,21
8	1,5	1,36	1,11	0,98
9	1,4	1,2	0,93	0,71
10	1,3	1,05	0,75	0,51
11	1,1	0,85	0,55	0,33
12	0,9	0,65	0,35	0,15
13	0,73	0,4	0,23	0,03
14	0,5	0,16	0,08	-
15	0,3	-	-	-
16	0,13	-	-	-
17	0,03	-	-	-
18	-	-	-	-

compounds that can prevent oxidation and reduction in skin tissue caused by microorganisms or other oxidizing compounds (Inya *et al*, 2008).

Based on the statistical analysis carried out, namely the Levene's Test of Equality of Error Variances analysis, the value of sig. 0.945> 0.05 indicates that the data obtained comes from the same variance, indicating that the data is homogeneous. One Way ANOVA analysis using the F test and obtained significant results 0.000 <0.05 which indicates that the variable formula affects the percentage of healing. Tukey test showed that the four treatments showed significantly different treatments. It shows that the treatment that has the most speed effect on wound healing in rabbits was extract 30%, 20%, 10% and most recently without botto'-botto' leaves extract. This means that in botto'-botto' leaves extract contains active substances that can accelerate the wound healing process, such as tannin, phenols, flavonoids, saponins, steroids and essential oils of alpa-pinene, cadinene, campfer, limonene, beta-caryophyllene and candinol isomers (Inya-Agha, *et al*, 2008). These secondary metabolites have anti-inflammatory (Banno, *et al*, 2005), antibacterial and anti-allergic effects (Tan *et al*, 2017), which can reduce the time of inflammation in the wound, thereby accelerating wound healing.

CONCLUSION

Based on the characteristics of the patch preparation, it showed that the formula with the best quality was formula III with 30% botto'-botto' leaves extract. The wound healing effect of incision wounds in rabbits showed that 30% botto'-botto' leaves extract had the fastest effect in the wound healing process compared to 10% and 20% extracts.

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