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The Effectiveness of the Antiinfamation Combination Gel of Okra Fruit (*Abelmoschus esculentus*) Extracts and Shallots Extract (*Allium cepa* L.)

Sara Nurmala*, Moerfia, Santi Novianti

Program Studi Farmasi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Pakuan
*Email: sara.nurmala@unpak.ac.id

Abstract

Wound is a condition that is the destruction of the unity or network components, which are specifically found the substance of tissue damaged or lost, either damage the continuity of the skin, mucous membranes and bones or other organs. Wound healing is a very complex process involving many cells. The process of wound healing is portrayed as occurs in biological surgical wound occurred. Several phases of wound healing are: the process of inflammation (inflammatory), the process of cell multiplication (proliferation) and cell maturation process (maturase). Okra is a plant that allegedly came from Southeast Asia. Okra has grown in many countries in almost all over the world. In the UK okra known as the Lady's Finger, in India it is called by the name of Bhindi while the United States called Gumbo. In Indonesia, this plant also has a local name that is Rabamea (Bima), Coffee Java (Java), Arabian Coffee (Sulawesi), Hoinu (Southeast Sulawesi), but better known by the name of okra. This study aims to determine the effective concentration and determine the length of time of wound healing using a combination gel okra fruit extract (*Abelmoschus esculentus*) and shallots (*Allium cepa* L.) as an anti-inflammatory against cuts in male white rats (*Sprague-Dawley*) with a length of observation for 9 days. This study uses Betadine® ointment as a positive control. The results showed the combination of fruit extract gel okra 6% and shallots 2% contained in the formula 3 most effective as an anti-inflammatory against cuts in male rats by administration for 6 days.

Keywords: Anti-Inflammatory, Gel, Okra, Shallots, Cuts Wound

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■ Introduction

Wound is a condition that is the damage to the unity or component of the network, where specifically there is a damaged or missing tissue substance, both damage to the continuity of the skin, mucous membrane and bone or other body organs. Wound healing is a very complex process

involving many cells. The process of wound healing is described as occurs in biological surgical wounds that occur in several phases of wound healing namely: the process of inflammation (inflammation), the process of cell multiplication (proliferation) and the process of cell maturation (maturase) [1].

Inflammation is a local tissue reaction to infection or injury and involves more mediators. Inflammation has a fairly high incidence rate, where inflames can be caused by trauma an illness [2]. Inflammation can be treated with alternative medicine using herbal plants, one of which is okra and shallots. Okra (*Abelmoschus esculentus*) is the only vegetable crop of significance in the Malvaceae family and is very popular in the Indo-Pak subcontinent. extract okra used as a good medicine for alzhemeirs disease [3]. Other plants that can be used for wounds are shallots. Shallots (*Allium cepa* L.) Is one of the plants originating from the countries of Iran and Pakistan which are then cultivated in cold, sub-tropical and tropical regions. Shallots are not only used as cooking spices, they are also believed to be used as medicines to heal wounds [4]. According to previous researchers 70% ethanol extract of shallots with a concentration of 20% can be used to heal wounds in male rats [5]. This study was conducted on the effectiveness test of anti-inflammatory gel combination okra and shallots extracts against cut wounds in male white rats. The choice of a combination of okra and shallots because seeing both plants has a synergistic effect on wound healing and it is hoped that the combination gel of okra and onion will provide more effective results on wound cuts in mice [6].

■ Materials and Methods

The materials used in this study include: Glassware, hair shavers, No. 11 surgical blade, wipes, scissors, test cages, glassware, okra and shallots from BALITTRO, Ethanol 96%, Ethanol 70%, betadine ointment, aquadest, magnesium, hydrochloric acid, Mayer reagents, Bouchardat reagents, Dragendorff reagents, $FeCl_3$ 1%, hydroxypropyl methylcellulose, triethanolamine, propylenglycol, phenoxyethanol, and male rats (Sprague-Dawley).

Plant Determination

Determination of plants in this study was carried out to ensure that the raw materials used in the study were correct and homogen. Plant determination is carried out at the Biological Research Center, Indonesian Institute of Sciences (LIPI) Kebun Raya, Bogor.

Preparation Simplicia of Okra Fruit

Prepare 10 kg of fresh okra, cleaned from dirt (wet sorting), then washed with running water until clean, then drained to disappear the remaining washing water. Clean okra fruit, dried in an oven with temperature (35 - 40 °C) until the okra is dry. Then the okra is sorted dry to remove the remaining impurities that are still attached to the okra fruit. The dried simplicia is then grinded to a powder simplicia and sifted with a mesh 40 sieve, then weighed to get the final weight of the simplicia. Simplisia powder is stored in a tightly closed container.

Preparation Extract of Okra Fruit

The Okra Fruit simplicia powder was extracted using maceration method. The Okra Fruit simplicia powder was weighed as much as 500 g and then put into a dark bottle. Add 5000 mL 96% ethanol macerated for 3 × 24 hours, each day the extract is filtered and collected. The waste is added to the solvent again and left for 24 hours, carried out for 3 days. The extract obtained was concentrated using a *rotary vacuum evaporator* at temperatures between 60-70°C until a thick extract is obtained.

Preparation of Shallot Extract

Extraction from shallots bulbs was carried out by maceration method. As much as 300 g bulbs of shallots are cleaned and peeled then the shallots are diced and then mashed using a blender juice. The shallots juice is then macerated repeatedly with 70% ethanol for 3 times each for 24 hours. The extract obtained is then evaporated and concentrated with a low pressure evaporator at 65°C.

Yield Simplicia

The yield simplicia that has been obtained is calculated using the function 1.

$$\text{Yield Simplicia} = \frac{\text{Simplicia weights obtained}}{\text{Simplicia Initial Weight}} \text{x} 100\% \qquad \text{(function 1)}$$

Yield Extract

The yield extract that has been obtained is calculating using the function 2.

$$\text{Yield Extract} = \frac{\text{Extract weights obtained}}{\text{Initial Weight}} \text{x} 100\% \quad \text{(function 2)}$$

Determination of Water Content

Determination of water content is done to determine the water content contained in okra fruit extracts using the gravimetric method. The empty cup is roasted first, then cooled and weighed. A total of 2 g of sample was put into a cup that was known to weigh. Then the cup is put into the oven at 105°C until a fixed weight is obtained. Calculation of water content is based on wet weight using the function 3.

$$\%\, Water\ Content = \frac{B-C}{B-A}\ x\ 100\% \quad \text{(function 3)}$$

Information :

A = Weight of the empty cup (g)
B = Weight of the cup + sample before drying (g)
C = Weight of the cup + sample after drying (g)

Determination of Total Ash Content

As much as 2 g of thick extract that has been weighed carefully, put into crushed poselin that has been incanded and tamed, then flattened. Crushes incandescent slowly until the charcoal runs out, incandescent is carried out at a temperature of 600 °C for 3 hours then cooled and weighed until a fixed weight is obtained. Ash content is calculated on the dried material [7].

Phytochemical Screening

Phytochemical screening of thick extracts of Okra fruit and shallots includes examination of flavonoid, alkaloid, saponin, and tannin compounds.

Identification of Flavonoid

As much as 5 g of thick extract added with Mg powder and added concentrated HCl, if orange, red and yellow are formed, the positive sample contains flavonoids [8].

Identification of Alkaloids

0.5 g of thick extract was added with 1 mL of 2N hydrochloric acid and 9 mL of distilled water, heated on a water bath for 2 minutes, cooled and filtered. The filtrate obtained was used for the alkaloide test, 3 tubes were taken as follow :

0.5 mL of filtrate were added. In each test tube:

a. Added 2 drops of Mayer reagent
b. Two drops of Bouchardat reagent are added
c. Added 2 drops of Dragendorff reagent

Alkaloids are positive if red or orange occur in Dragendroff reagents, brown color in Bouchardat reagents, and white deposits occur in Mayer reagents [7].

Identification of Saponin

0,5 g of thick extract was put into a test tube, then added 10 mL of hot water, cooled and shaken vigorously for 10 seconds. If a stable 1-10 cm high foam is formed not less than 10 minutes and does not disappear with the addition of 1 mL of HCl 2N indicates the presence of saponins [7].

Identification of Tanin

As much as 1 g of thick extract is added 1-2 drops of 1% iron (III) chloride reagent. If there is a blackish blue or blackish green, while condensed tannins will cause a brown color. A solution of 1% gelatin in 10% sodium chloride causes deposition in tannin solution [9].

Formula Gel Combinations of Okra and Shallot Extracts

The formula for the gel preparation combination of Okra fruit extracts and shallots can be seen in Table 1.

Table 1. Combined Gel Formula Extract Okra Fruit and Shallot Extract

Raw Material	F0 (%)	F1 (%)	F2 (%)	F3 (%)	F4 (%)
Okra fruit extract	0	2	4	6	8
Shallots extract	0	4	3	2	1
HPMC	2.5	2.5	2.5	2.5	2.5
Trietanolamin	3	3	3	3	3
Propilenglikol	15	15	15	15	15
Fenoksietanol	0.5	0.5	0.5	0.5	0.5
Aquadest	ad 100	ad 100	ad 100	ad 100	ad 100

Source: Modified from [10]

Preparation of Combination Gel

All ingredients are weighed, then the distilled water is heated to 70°C. A number of HPMC is mixed into water with a temperature of 70 °C let stand until it expands and forms a gel. The mixture is added with other ingredients such as phenoxyethanol and propylenglycol, then mix a number of extracts of okra fruit and onion extract and then add the remaining distilled water crushed until homogeneous.

Gel Evaluation

Organoleptic Test of Gel.

Organoleptic observations of gel preparations include changes in color, odor, and texture [11].

Homogeneity test preparation.

a certain amount of preparation is applied to two pieces of glass or other suitable transparent material, the preparation must show a homogeneous arrangement with no visible coarse grains.

Measuring the pH

Measuring pH of the gel is done using pH paper. It is done by weighing 10 g of the preparation dissolved in 50 mL aquadest in a beaker glass, adding aquadest to 100 mL then stirring until evenly distributed. The solution was measured using standardized pH paper.

Testing effective of antiinflamation Combination of Okra Fruit Extract and Shallot Extract

Strain male white rats (Sprague-Dawley) prepared 30 tails then weighed one by one the body weight of rats, calculated on average, then divided into 6 large groups of each group of 5 rats, placed in a container covered with husk and covered with wire mesh, given food 2 times a day drinking ad libitum, and acclimatization for 1 week in a cage.

Treatment of Experimental Animals

Experimental animals that have been acclimated for 1 week. All rats used, sliced along 1,5 cm and a depth of 0,7 mm on the back are parallel to the back area of the rat using surgical blade No. 11 that has been sterilized previously [12]. The hair of the mice around the incision area is shaved before the incision is made, then cleaned with 70% alcoholic cotton and 5% Emla anesthetic ointment evenly applied to the area to be injured. Each group was smeared with a combination mixture of okra fruit wound extract and thin shallots extract ± 0.2 g gel after making the wound.

Anti-inflammatory Testing

Tests were carried out on 30 male white rats divided into 6 groups, each of which were 5 rats per perlakua group. The division of groups can be seen in Table 2.

Table 2. Treatment Test

Group	Treatment	Total Rats
Control (+)	Given group Betadine®ointment	5
Control (−)	Groups that are given a base gel	5
Formula 1	The group was given a combination of fruit extract gel okra concentration of 2% and shallots extract 4%	5
Formula 2	The groups were given a combination of okra fruit gel with a concentration of 4% and shallots extract 3%	5
Formula 3	The group was given a combination of fruit extract gel okra 6% concentration and 2% shallot extract	5
Formula 4	The groups were given a combination of 8% okra fruit gel and 1% onion extract	5

The treatment is done 5 minutes after the wound is made, the gel is applied twice a day morning and evening until the wound heals.

Parameters

The parameters measured and the changes observed were shortening of the wound (mm) for closure to the wound, [12]. Then by scoring the wound closure in male rats. The following score is the condition of the wound:

1. Red sores, severe edema, wet, open sores.
2. Red sores, mild edema, wet, open sores.
3. Pale red sores, slightly dry wound edges, narrow wounds
4. The wound edge is rather dry, the wound is narrowed
5. Narrow wounds, hard wound edges, scabs have formed.
6. The wound is narrowing and shallow, the scar becomes soft, scab formed.
7. Visible scab scars, soft scars, narrow wounds.
8. The wound has closed, the former scab is gone.

Data analysis

The research data were analyzed using a Completely Randomized Factorial Pattern Design and Duncan's further tests to see differences between treatments. In this experiment 6 group treatments were carried out with 6 repetitions. RAL Factorial Pattern is an experiment of more than one factor that can be applied directly to all experimental units. If the unit used is relatively homogeneous, then it is called a two-factor design in a factorial completely randomized design (CRD). Normal and homogeneous data distribution is processed by the ANOVA test method followed by the Duncan test [13]. The analysis was carried out using the SPSS program.

■ Results and Discussion

Plant Determination Results

The plants used in this study were okra and shallots obtained at the Research Institute for Medicinal and Aromatic Plants (BALITTRO). Results of Plant Determination conducted at the Center for Plant Conservation of the Indonesian Research Institute of Sciences (LIPI) Botanical Gardens, Jalan Ir. H. Juanda No. 13, P.O.BOX 309 Bogor 16003, Indonesia. Stating that the plants used in this study were Okra (*Abelmoschus esculentus* L Moench) and Shallot (*Allium cepa* L.).

Yield Simplicia of Okra Fruit

Okra fruit used is fresh green okra fruit. From 10 kg of okra fruit obtained 1510 g of simplicia powder with a simplicia yield of 15.1%.

Yield Extract of Okra Fruit

Simplisia obtained okra then extracted by maceration method using 96% ethanol solvent. The results of maceration of okra fruit extract from 500 g of simplicia powder in 5000 mL of solvent produced thick brown extract of 114.70 g with a yield of 7.5% extract.

Yield Extract of Shallots

The results of maceration of shallot extract from 300 g of fresh material in 3000 mL of solvent produced thick brown extract that has a distinctive odor of 92.5 g with a yield of 30.8% extract.

Determination of Water Content

Determination of water content was carried out using Gravimetri method which was carried out in duplo with the results of the water content obtained in okra fruit extracts of 4.0367% and shallots extracts of 3.125%. These levels meet the general requirements of not more than 10% [7]. Determination of water content aims to provide a limit of water content that can still be tolerated to maintain the stability of the extract.

Determination of Total Ash Content

Determination of ash content aims to provide an overview of mineral content and external contamination contained in okra fruit extracts and shallots extract. The condition for the ash content in the extract is less than 5% [7]. The results of the ash content obtained by okra were 1,011% and the shallots 3.95% then the results meet the requirements.

Phytochemical of Okra and Shallots

Phytochemical tests include tests of flavonoids, alkaloids, saponins and tannins. The results can be seen in Table 3.

Table 3. Phytochemical identification test results okra extract and shallots extract

No	Phytochemical tests	okra extract	shallots extract
1	Flavonoid	+	+
2	Alkaloid	+	+
3	Saponin	+	+
4	Tannin	+	+

Information :
(+) : Positive yield
(-) : Negative yield

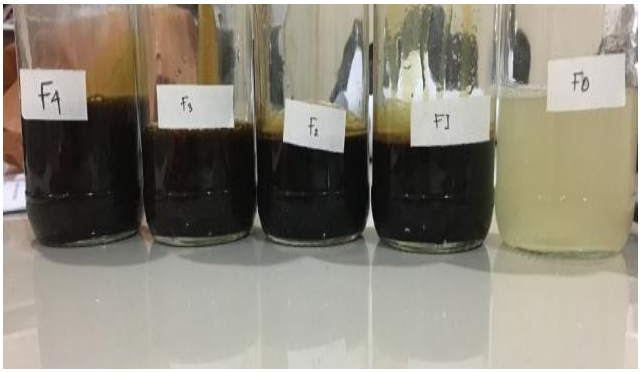

Figure 1. Image of gel preparations for combination of okra extract and shallot extract

Evaluation of a combination gel of okra and shallots extract.

Organoleptic test is done visually, directly seen the color, odor and shape of the gel combination that has been made. The results of organoleptic testing of the four gel preparation formulas with the addition of a combination of extracts obtained the results of a blackish brown color, aromatic distinctive aroma. The homogeneity test of the four combination gel formulas was declared homogeneous. The pH test was carried out on a gel base combination of okra fruit and onion and the results of the examination showed that the pH of the combination gel in formulas 1, 2, 3 and 4 obtained a pH of 6, this shows that the combination gel preparations are still in the range of pH requirements for the skin that is 4.5 - 6.5 [14].

Data Analysis Results

The results of observing the wound healing wound score data in Sprague-Dawley male white rats in the 1st to 6th treatments are presented in Table 4.

Table 4. Observation results of mean scores of wound conditions

Treatment	Observation Day to-									Average SD
	1	2	3	4	5	6	7	8	9	
Control (-)	1	2	2.2	3	4	5	6	7	8	4.2^{a}±2.449
Formula 1	1	2.2	3	4.2	5	5.6	6.7	8	8	4.85^{b}±3.082
Formula 2	1	2.8	3.8	4.2	5	6	7.2	8	8	5.1^{c}±2.880
Formula 3	1	3.4	5.4	6.4	7.2	8	8	8	8	**6.1^{e}±3.130**
Formula 4	1	3.2	4	5.8	6.8	7.4	8	8	8	5.8^{d}±3.193
Control (+)	1	4	6	7	8	8	8	8	8	**6.4^{e}±2.455**
Average	1±0	2.9^{b}±1.414	4.0^{c}±1.527	5.1^{d}±2.828	6^{e}±1.732	6.6^{f}±1.5	**7.3^{g}±1**	**7.8^{g}±0.408**	**8^{g}±0**	

Based on the observations of the average score in Table 4, it is known that the treatment groups of formula 1, formula 2, formula 3, and formula 4 show a real influence with control (-) on healing wound. In the control treatment (-) the longest wound closure occurs because it is only given a gel base without the active ingredient. The control (+) shows the fastest healing in the wound. The results of the test on the combination preparation showed that the fastest wound healing was the treatment of the formula 3 group gel combination of 6% okra extract and shallots 2% compared the treatment of other formula groups, formula 3 is almost equivalent to the control (+). Judging from the time of healing that has been observed, the wound is classified as an acute wound or type of wound during the healing period, then the wound will still heal and return to normal conditions even without the treatment process, but the comparison is the duration of healing time and the speed of complete wound closure.

Table 5 shows the same superscript letters between the control group (+) and the 3 formula group treatments, seen from the duration of treatment the same superscript letters were shown on the sixth day. This can be interpreted that the combination gel of okra fruit extract and onion extract can help the coagulation process, the coagulation process is a complex process, in which blood forms clots to close and heal wounds, and stop bleeding [15]. In each treatment group, formula 3 showed an anti-inflammatory effect that was almost the same as the control (+), then followed by the treatment group formula 4, formula 1 and formula 2. The slowest wound healing effect was the treatment group (-) with a long time 9 days. Research [5] showed that 70% ethanol extract of shallots with a concentration of 20% could be used to cure rat wounds within 9 days. Okra extract ointment with a concentration of 6% gives effective results as anti-inflammatory [16].

Table 5. Results of observation of cut wound scores

Groups	Day-to-day observations								
	1	2	3	4	5	6	7	8	9
Control(-)	1a	2b	2,2b	3c	4e	5fg	6h	7j	8k
Formula1	1a	2,2b	3c	4,2e	5fg	5,6gh	6,7i	8k	8k
Formula 2	1a	2,8c	3,8de	4,2e	5fg	6h	7,2j	8k	8k
Formula 3	1a	3,4cd	5,4g	6,4i	7,2j	8K	8k	8k	8k
Formula 4	1a	3,2cd	4e	5,8h	6,8ij	7,4jk	8k	8k	8k
Control (+)	1a	4e	6h	7j	8k	8k	8k	8k	8k

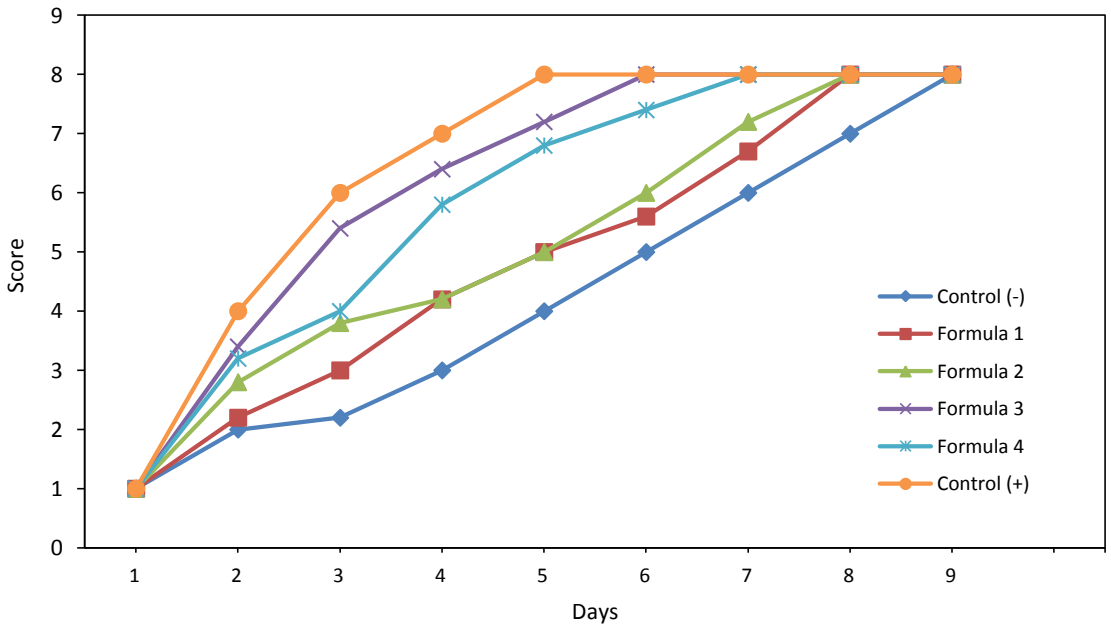

Figure 2. Graph Observation Results Score Average Wound Conditions

Figure 2 show that In the treatment group of formula 3 which contains 6% okra and 2% shallots show that formula 3 is the best formula in wound healing compared to formula 1, formula 2 and formula 4. It can be interpreted that formula 3 with 6% okra fruit content and shallots 2% has the potential to be very active in wound healing because okra and shallots contain secondary compoundsflavonoids. Flavonoid compounds are thought to provide anti-inflammatory activity, because flavonoid compounds work by inhibiting an important phase in the biosynthesis of prostaglandins, namely in the cyclooxygenase enzyme pathway which works as an anti-inflammatory.

Conclusion

Based on the research on the Effectiveness of Anti-Inflammation Test of the Combination of Gel Extract Okra Fruit and Shallots Extract on cut wounds in male white rats, it can be concluded that the combination gel of 6% okra fruit extract and 2% shallots contained in formula 3 is most effective as an anti-inflammatory against cuts in male white rats and Obtained a long time of treatment from the combination of 6% okra fruit extract gel and 2% shallots as an anti-inflammatory against cuts in male white rats for 6 days.

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