

Anti-inflammatory Activity of the Gel Containing a Combination of Neem Leaf Extract and Shallot Extract

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Abstract

Neem leaves (*Azadirachta indica* A. Juss) and shallots (*Allium cepa* L) contain flavonoids that are effective as antioxidant, antibacterial, and anti-inflammatory. The aim of this study was to determine the effective concentration and the duration of treatment of anti-inflammatory activity of the gel containing a combination of neem leaf extract and shallot extract. These substances have similar effect, and it is believed that these combination may effective as anti-inflammatory at small doses to reduce the side effects. The animals were randomly divided into 6 groups with 5 rats per group. The rats were wound-induced by incising its back (length 1.5 cm, depth 0.7 mm) and were observed for 8 days. Parameters measured were wound's closure, color, and healing process. Statistical analysis showed that the most effective concentration as an anti-inflammatory was the formula 1 (neem leaf extract 3.125% : shallots extract 4%) with the fastest healing time was 5 days. Thus, this formula is one of the effective treatment options for healing wounds.

Keywords: neem leaves, shallots, anti-inflammatory, combination, gel

Introduction

Indonesia has approximately 30,000 plant species, including the neem leaf plants (*Azadirachta indica* A. Juss) and shallots (*Allium cepa* L). Neem leaf is one of the *Meliaceae* family, which is used in hereditary medicine for more than 4000 years. It contains paraisin, alkaloids, flavonoids, tannins, saponins, and essential oil components that contain sulfide compounds. Neem leaf has an anti-inflammatory, antibacterial, antifungal, and antioxidant effects.¹

Shallot is a member of the *Liliaceae* family. This plant contains quercetin which has anti-inflammatory effect. Quercetin is one of the

active ingredients of the flavonoid that can be used as an anti-inflammatory drug, including infection in the open wound.²

Injury is the loss or damage in a portion or in a component of the body tissue. Combination products that consist of two or more medicinal elements in one dosage unit is believed more effective rather than only a single drug to heal injury. The results of 70% ethanol extract of neem leaves (concentration of 6.25%) are effective as incision wound healers on male Swiss-Webster mice.³ Hakiem has tested 70% ethanol extract of shallot as a wound healer in Sprague-Dawley male rats, showing that ethanol extract of shallots (concentration

20%) has an effect on wound healing.⁴ Regarding the efficacy of each plant, the authors are interested in conducting research on topical effectiveness of anti-inflammatory test from a combination of neem leaves and shallots in rats.

Methods

Neem Leaf Extraction

A total of 500 g of dried leaves powder was extracted using 5000 mL of 70% ethanol as the solvent. The extraction was repeated 3x24 hours. The extract obtained was concentrated using a rotary vacuum evaporator at 60-70°C to obtain a thick extract.

Shallots Extraction

A total 1 kg of shallots was cleaned, stored at 4°C, and mashed using a blender. Shallots juice was then macerated repeatedly with 3000 mL of 70% ethanol for 3x24 hours. The macerates were filtered using a Buchner funnel. The extract then evaporated and concentrated using a rotary vacuum evaporator at 65°C.⁴

Water Content Determination

The determination was carried out using gravimetric methods. The empty cup was roasted, cooled, and weighed. A total of 2 g of sample was put into a cup, and dried in the oven at 105°C until a fixed weight was obtained.

Ash Content Determination

A total of 2g thick extract was weighed carefully, put into a crucible, flattened slowly until the charcoal runs out. The crucible was cooled then weighed constantly. Total ash content was calculated against the weight of the test material expressed in % w/w.⁵

Phytochemical Screening

Phytochemical screening was carried out to determine the presence of flavonoids, tannins

and saponins on the neem and shallots extracts.⁶

Formulation of Gel Combination

Each gel contained of neem and shallots extracts and formed for 100 g. The following formulation is shown in Table 1.⁷

Gel Formulation

All materials were weighed and heated with 70°C distilled water. HPMC is put into water at a temperature of 70°C; stirring slowly until it expands and forms a gel. The mixture was added with other ingredients such as triethanolamine, propylenglycol, and phenoxyethanol, mixed with a number of neem leaf extracts and shallots extract according to the combination formula, then was added the remaining aquadest water, mixed until homogeneous.

Animals Treatment

Thirty males Sprague-Dawley white rats. The rats were weighed and calculated its coefficient of variation. The rats were divided into 6 groups and acclimatized for 1 week by following ethical standard procedure for animal research. After acclimatized, the experimental animals were given treatment according to Table 2. Before the treatment of animal injuries, rats were anesthetized. During treatment, paracetamol was given orally to reduce pain of the wound. Wound observation was observed for 8 days and the scoring of the wound was calculated daily. Treatment gel dosage can be seen in Table 2.

Parameter and Wound Score

The wound length and the closure of a scratch were measured and observed.⁸ The scores of the parameter are :

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.

Table 1. Formulation Gel Combination Neem and Shallots

Material Name	F0 (%)	F1 (%)	F2 (%)	F3 (%)	F4 (%)
Neem Leaf Extract	0	3.125	6.25	12.5	25
Shallots Extract	0	4	3	2	1
HPMC	2.5	2.5	2.5	2.5	2.5
Triethanolamine	3	3	3	3	3
Propylenglycol	15	15	15	15	15
Phenoxyethanol	0.5	0.5	0.5	0.5	0.5
Distilled Water	Ad 100				

Table 2. Treatment Gel Dosage

Group	Treatment	Number of Rat
Positive Control	Betadine Ointment	5
Negative Control	Base Gel	5
Formula 1	Neem:Shallots (3.125%:4%)	5
Formula 2	Neem:Shallots (6.25%:3%)	5
Formula 3	Neem:Shallots (12.5%:2%)	5
Formula 4	Neem:Shallots (25%:1%)	5

- The edge of the wound is rather dry, the wound is narrowed.
- Narrow wounds, hard wound edges, scabs have formed.
- The wound is narrowing and shallower, the scar becomes soft, formed scab.
- Visible scab scars, soft scars, narrow wounds.
- The wound has closed, the former scab is gone.

Data analysis

Data were analyzed using a Completely Randomized Factorial Pattern Design and Duncan's for further tests. In this experiment 6 treatments were carried out with 5 repetitions. RAL Factorial Pattern is a two-factor experiment that can be applied directly to all experimental units. If the experimental unit used is relatively homogeneous, then it is called a two-factor design in a completely randomized design (RAL) factorial pattern of normal and homogeneous data distribution processed by ANOVA test method followed

by Duncan test.⁹

Results and Discussion

Neem leaves and Shallots

Yield of extract neem leaf powder is 22.18% and yield of extract shallots is 30.8%.

Water Content

Determination of water content was 3.21% neem leaf extract and 3.42% shallots extract then these results fulfilled the requirements.

Ash Content

Determination of ash content of neem leaf extracts obtained an average of 3.18% and shallots extract obtained 3.35% then the results fulfilled the requirements. Ash content in a food can be influenced by several factors that can affect such as species, soil nutrient conditions, environmental factors such as rainfall, soil and pH.

Phytochemical Screening

Phytochemical screening is shown in Table 3.

Table 3. Treatment Gel Dosage

Test	Neem	Shallots
Alkaloids	+	+
Flavonoids	+	+
Tannin	+	+
Sapponin	+	+

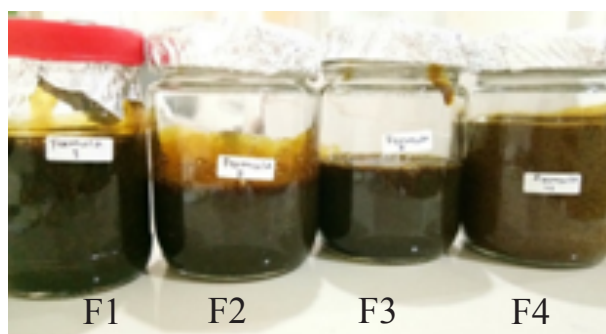


Figure 1. Gel Combination Extract Neem Leaf and Shallots Extract

Gel Formulation Combination of Neem Leaf Extract and Shallot Extract

The gel preparation and combination of neem leaf and shallots extract picture is shown in Figure 1. The color of formula I the preparation was brown and in formula II, formula III, and formula IV was blackish brown. It can be interpreted that the higher the concentration the more concentrated for brown color.¹⁰

Wound observation results

The results of observing the wound score at fifth day can be seen in Table 4 below :





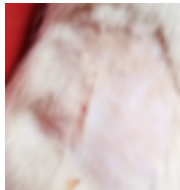

The treatment groups of formula I, formula II, formula III, and formula IV showed a significant effect with control (-) on wound healing. In the control group treatment (-) the longest wound closure occurred because only given a gel base without active substances. The (+) control showed the most rapid closure of the wound in the Sprague-Dawley male white rat. The test results showed that the fastest wound healing was the treatment of group formula I gel combination of neem leaf extract 3.125% and shallots extract 4%.

This can be interpreted at a concentration of 3.125% neem leaf extract and 4% shallots extract has the potential to be very active in wound healing and can also be interpreted that shallots extract has a higher effect compared to neem leaf extract on anti-inflammatory.

Allegedly because of the activity of high quercetin founded in shallots, quercetin is one of the flavonoid compounds that is thought to provide anti-inflammatory activity.¹¹ Flavonoid compounds can inhibit inflammation by inhibiting the enzymes cyclooxygenase and lipooxygenase enzymes during arachidonic acid metabolism, so that inflammatory mediators of leucotrienes, histamine, bradykinin, thromboxane, and prostaglandins are inhibited.¹²

Flavonoids work by inhibiting an important phase in the biosynthesis of prostaglandins, namely the cyclooxygenase enzyme pathway. Gel is preferred for topical application due to more stability and better application property.¹³ Provision of herbal ingredients topically has been known to provide more

Table 4. Treatment Gel Dosage

Group	Observation Day 5
Negative Control	
Positive Control	
F1	
F2	
F3	
F4	

optimal results in wound healing, especially in accelerating wound contractions. This is due to topical use, more drug compounds accumulated on the wound side.¹⁴ The results of research on wound healing of rat wound by providing topical gel combination of neem leaves and shallots in various concentrations of concentration tests have shown a maturation phase where the wound surface has been completely closed on the fifth day, this study

is better than research on topical preparation of shallots extract without combination with the 10 day wound healing process.¹⁵

Data analysis

The Duncan test results show that all combinations of Neem leaves (*Azadirachta indica* A. Juss) and shallots (*Allium cepa* L) have anti-inflammatory power. The combination of formula 3 is not significantly

different from the positive control and all combination formulas are significantly different from the negative control.

Based on the observations of the average score in Table 5, it is known that the treatment of groups of formula 1, formula 2, formula 3, and formula 4 showed a significant effect with control (-) on wound healing. In the control group treatment (-) the longest wound closure occurred because only given a gel base without active substances. The (+) control showed the most rapid closure of the wound in Sprague-Dawley male white rats. The test results showed that the fastest wound healing was the treatment of group formula 1 gel combination of neem leaf extract 3.125% and onion extract 4% compared to the treatment of other formula groups, formula I was equivalent to control (+). Judging from the time of wound healing that has been observed, including the type of acute wound or type of wound during the healing period, 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.

The strengths of this study are to get a combination of drugs that have synergistic work effects because the combination of two active substances that have been tested each has a good effect in relieving inflammation, and limitations of this study are limitations of experimental animals used because researchers are committed to doing reduce in the ethical use of experimental animals, the repetition of animals in each group is carried out to a minimum and researchers must really take care that the animals are not eliminated during the study period.

Conclusion

It can be concluded that gel containing a combination of neem leaf extract 3.125% and

shallots extract 4% have the most effective potential as an anti-inflammatory in male white rats. Duration treatment combination gel of neem leaf extract 3.125% and shallots extract 4% for curing the wound is 5 days.

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