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Anti-Inflammatory Activity Test of Ointment Preparations of Nutmeg Leaf Extract from Fak-Fak (*Myristica argentea* Warb) Against Burns in Male Wistar White Rats

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ABSTRACT
The fak-fak nutmeg plant (*Myristica argentea* Warb) is one type of spice plant native to Indonesia originating from fak-fak City. This nutmeg is included in the *Myristicaceae* family which can have secondary metabolite compounds in the form of alkaloids, tannins, flavonoids, saponins, and phenolics which have biological activity as an anti-inflammatory burn. This study aims to determine the anti-inflammatory activity and concentration of effective preparations. This study used test animals consisting of 15 male white rats which were divided into 5 groups, namely group I negative control (ointment base), group II positive control (bioplacenton) group III ointment extract of nutmeg leaves from fak-fak concentration 2%, group IV ointment extract of nutmeg leaves from fak-fak concentration 6% and group V ointment extract of nutmeg leaves from fak-fak concentration 10%. From the results of the research that has been done, the ointment preparation with 2% extract concentration shows inflammation healing activity against rats with wound shrinkage (0.676), ointment with 6% extract concentration (0.374) and for ointment with 10% extract concentration (0.313) while in the positive group shows inflammation healing activity with wound shrinkage (0.508) and negative group (0.755). From the results obtained, it can be concluded that the ointment preparation of nutmeg leaf extract from fak-fak (*Myristica argentea* Warb) is effective in healing inflammation, the most effective ointment preparation is an ointment preparation with 10% extract concentration.

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INTRODUCTION

Burns are injuries that occur when body tissues are exposed to direct heat such as chemicals, hot water, electricity or radiation and fire (Wahyudi and Agustina, 2018). Partial thickness burns distinguish this type of injury as it burns only the epidermal tissue while the

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dermis tissue remains intact. Total burns occur at the above level, where a portion of the dermis is burned, reducing the loss of body fluids and proteins (Usman *et al*., 2024).

Hemostasis, inflammation, proliferation and remodeling are all part of the wound healing process (Astuti, 2020). After tissue damage, the hemostasis phase occurs. Platelets are secreted by damaged blood vessels to prevent excessive blood loss. This is followed by the inflammatory phase, where neutrophils and macrophages phagocytize bacteria and debris, and the proliferation phase, where the formation of new tissues and blood vessels begins. Remodelling is the last stage of wound healing (Primadina *et a*l., 2019).

Local therapy, which aims to ensure the burn heals as quickly as possible, is a common treatment for burns. Many people use natural or herbal medicines because they can be bought without a prescription, can be concocted by themselves, are affordable, and medicinal plants can be grown by themselves (Usman *et al*., 2024). One of the plants that can be used as a traditional medicine is *Myristica argentea* Warb, also known as "Fak-fak nutmeg" by the Papuan people, originating from the city of Fak-fak, which has the original name henggi. All parts of the plant, including the pulp, seeds, mace, and leaves, can be used as medicine (Musaad *et al*., 2017).

The results of previous researchers showed that banda nutmeg leaves (*Myristica fragrans Houtt)* contain flavonoids, saponins, alkaloids, tannins, and terpenoids in phytochemical tests (Nasir, 2022). Flavonoid compounds work as anti-inflammatories by blocking capillary permeability, arachidonic acid metabolism, and lysosomal enzymes secretion. Lysosomal enzymes function as inflammatory mediators, which have the ability to prevent the proliferation of the inflammatory process. By stimulating collagen formation, saponin compounds help to increase tissue epithelialization, which allows the wound surface to be closed (Priamsari, 2019).

Based on this explanation, the researcher intends to make ethanol extract of nutmeg leaves from Fak-fak in an ointment preparation as an anti-inflammatory to treat burns in wistar male white rats, because there is no research related to testing the anti-inflammatory activity of ethanol extract of nutmeg leaves from Fak-fak in the form of ointments applied to burns of wistar male white rats.

METHODS

Tools and materials

The tools used are aluminum foil, erlenmeyer, filter paper, stirring rod, analytical balance, mice cage, mortar, stamper, iron plate, glass bowl, water bath, porcelain cup, shaver, ph meter, glass funnel, ruler, glass jar, beaker glass, dropper, ointment pot, solder, water bath. The materials used in this study are nutmeg leaves (*Myristica argentea* Warb), 70% ethanol, vaselin album, adaps lanae, distilled water, and bioplacenton. In this study, 24 male white rats weighing 150-200 grams were used as experiment animals.

Process of Sample Extraction

Sample extraction using maceration method. Previously, fresh fak-fak nutmeg leaves were taken, cleaned with running water and dried using an oven at 50°C. After drying, the

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size was reduced using a blender until it became powder. Dry powder of 500 grams was put into a maceration vessel and then macerated for 5 days with 70% ethanol solvent as much as 2L, after which it was filtered using filter paper and obtained filtrate 1 and pulp 1. The pulp was re-soaked using 70% ethanol solvent with the same amount of solvent for 2 days (occasionally stirred), then filtered and obtained filtrate 2 and pulp, then filtrate 1 and 2 were combined into one, then evaporated with a water bath until a thick extract was obtained.

Phytochemical Screening Test

Flavonoid Compounds

In a test tube, a sample of 2 ml of fak-fak nutmeg leaves was placed. Then, 2 to 3 drops of Pb II acetate were added. The formation of a yellow precipitate indicates the presence of flavonoid compounds (Sulistyarini *et al*., 2016).

Alkaloid Compounds

A sample of 2 ml was put in a test tube, then given mayer and bouchardat reagents as much as 2-3 drops. The presence of alkaloid compounds is characterized by the formation of white or yellow precipitates and brown precipitates (Rohmania *et al*., 2024).

Saponin Compounds

A total of 2 ml of fak-fak nutmeg leaf extract (*Myristica argentea* Warb) was put into a test tube and added 3-4 drops of distilled water and shaken. The positive result of saponin is characterized by the formation of foam (Astika *et al*., 2022).

Taninn Compounds

A 2 ml sample of fak-fak nutmeg leaves was put in a test tube and then added with FeCl3 reagent. positive results contain tannins when a dark blue or blackish color is formed (Saadah and Tulandi, 2020).

Formula

Table 1. Formulation of Nutmeg Leaf Extract of Fak-fak Origin (*Myristica argentea* Warb)

Ointment Formulation	Ointment Concentration		
	2%	6%	10%
EEDPAF	0,6 g	1,8 g	3 g
Ointment Base	29,4 g	28,2 g	27 g
m.f.ungt	30 g	30 g	30 g

Information :
EDPAF : fak-fak origin nutmeg leaf extract
m.f.ungt : mix and make the ointment

Ointment Making

The method of manufacture is to weigh all the ingredients according to the formulation then vaselin album and adaps lanae are crushed in a mortar until homogeneous, then the ethanol extract of nutmeg leaves from fak-fak is added according to the formulation where 0,6 g for 2% concentration, 1,8 g for 6% concentration and 3 g for 10%

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concentration. Grinded in a mortar until homogeneous. After homogeneous, it was put into the ointment pot and then labeled (Paju *et al.*, 2013).

Test Animal Preparation

In this study, 15 wistar male white rats were used as test animals with a body weight of 150-200 grams. Previously acclimatized for 7 days before the study, so that the test animals are accustomed to the new environment and treatment placed in a cage and fed enough every day.

Burn Preparation

The process of making burn wounds, before being treated, rats are anesthetized using ketamine, the recommended dose is 50 mg/kg BW (Rahman, 2024). Intramuscularly, the hair on the back of the rat was shaved with a diameter of 3 cm. Heat a 2 cm diameter metal on a bunsen flame for 1 minute and then attach it to the rat's back for 5 seconds (Ghofroh dalam Rahmadani *et al.*, 2021).

Observation of Wound Area Ratio

Wounding was performed on day 0 and the wound healing process was observed daily for 14 days. Wounds on the back of mice will be treated 2x a day morning and evening. Before the mice wound is measured, the wound is cleaned with NaCl solution, then the mice wound is measured using ruler paper and then photographed, the data is taken by placing playfenl plastic over the mice wound and circularly following the wound area, given medicine and bandaged again. After 14 days, the wound area was calculated with Scion Image Beta 4.02 analysis software (Astuti *et al.*, 2021).

RESULTS AND DISCUSSION

The leaves of fak-fak nutmeg (*Myristica argentea* Warb) used in this study are fresh leaves as much as 2kg which are picked directly from fak-fak nutmeg trees with fresh green characteristics. The nutmeg leaves from fak-fak were dried using an oven at 50°C, then the dried leaves of fak-fak nutmeg were made into powder using a blender, then sieved using a sieve after which the extraction process was carried out using the maceration method by soaking the powder using 70% ethanol solvent for 5 days, solvent ratio 1: 4 (500 g: 2000 ml) then continued with remaceration for 2 days with the same solvent ratio. Evaporation of the extract of nutmeg leaves from Fak-fak water was carried out using a water bath at a temperature of 50 ° C until concentrated and alcohol-free. Based on the results of maceration carried out, from 500 grams of dry simplisia obtained a thick extract of 33 grams so that the extract yield obtained was 6.6%.

In this study, a phytochemical screening test was carried out to identify the content of compounds contained in the extract of nutmeg leaves from fak-fak (*Myristica argentea* Warb), aimed at active compounds of saponins, flavonoids and tannins. This test aims to ensure that saponin, flavonoid and tannin compounds are expected to affect burn wound healing in the ointment preparation to be made. The test results showed that the ethanol extract of nutmeg leaves from fak-fak (*Myristica argentra* Warb) positively contained saponin and flavonoid compounds. The test results can be seen in table 2.

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Saponin compounds stimulate collagen formation which plays a role in increasing tissue epithelialization so that it can close the wound surface (Syamsuhidayat dalam Priamsari, 2019). Flavonoid compounds work as anti-inflammatory by inhibiting capillary permeability and inhibiting arachidonic acid metabolism and inhibiting the secretion of lysosomal enzymes as inflammatory mediators that can inhibit the proliferation of the inflammatory process (Robinson dalam Priamsari, 2019). Tannins can help the wound healing process as they function as antioxidants and antimicrobials (Ningrum *et al.*, 2023).

Table 2. Phytochemical Screening Test Results of Nutmeg Leaf Extract from Fak-fak (*Myristica argentea* Warb)

Compound	Reagents	Results	Test Results
Flavonoid	Lead (II) Acetate	+	Yellow precipitate
Saponin	Aquadest	+	There is foam
Tanin	Iron trichloride ($FeCl_{3)}$	+	Dusky green
Alkaloid	Mayer	+	White precipitate
	Bouchardat	+	Brown precipitate

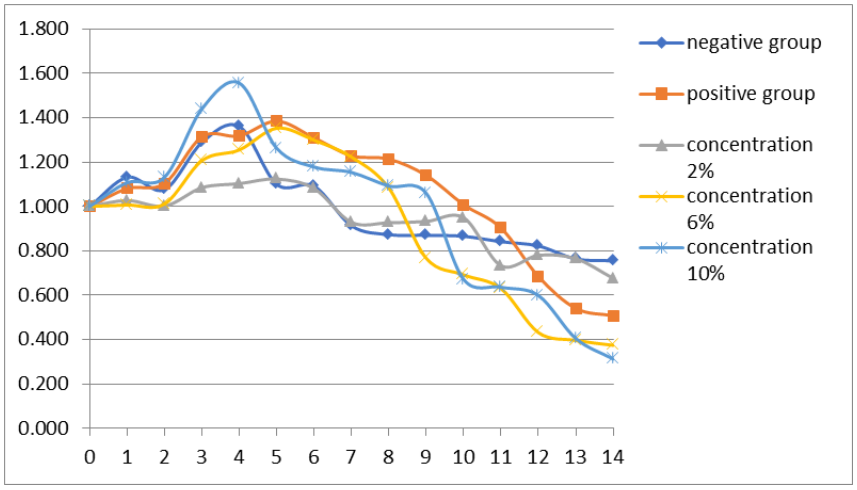

Figure 1. Wound Area Ratio Chart

Evaluation of the wound area ratio shows that all groups have a wound healing pattern that is not much different, where the wound size enlarges during the inflammatory phase then decreases gradually during the proliferative phase process. It can be seen in the graph that the negative group and the positive group experienced the peak of inflammation on different days where the negative group on day 4 and the positive group on day 5, as well as the 2% and 6% concentration groups experienced the peak of inflammation on day 5 while the positive group experienced the peak of inflammation on day 4 where the wound area increased due to swelling (tumor), pain (dolor) and redness due to dilated thin blood vessels (rubor). Furthermore, the proliferation phase will take place at the end of the inflammatory phase until the end of the third week. From day 6 to day 14, it can be seen in the graph that the wound area decreases when it reaches the peak of inflammation where

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on day 14 the negative group has a wound area of (0.755), positive group (0.508), 2% concentration group (0.676), 6% concentration group (0.374) and 10% concentration group (0.313). In this phase the body begins to build new tissue and close the wound layer with scar tissue.

The results of observations of changes in wound area can be seen in Figure 2 from day 0-14, it can be seen that the wound has begun to close.

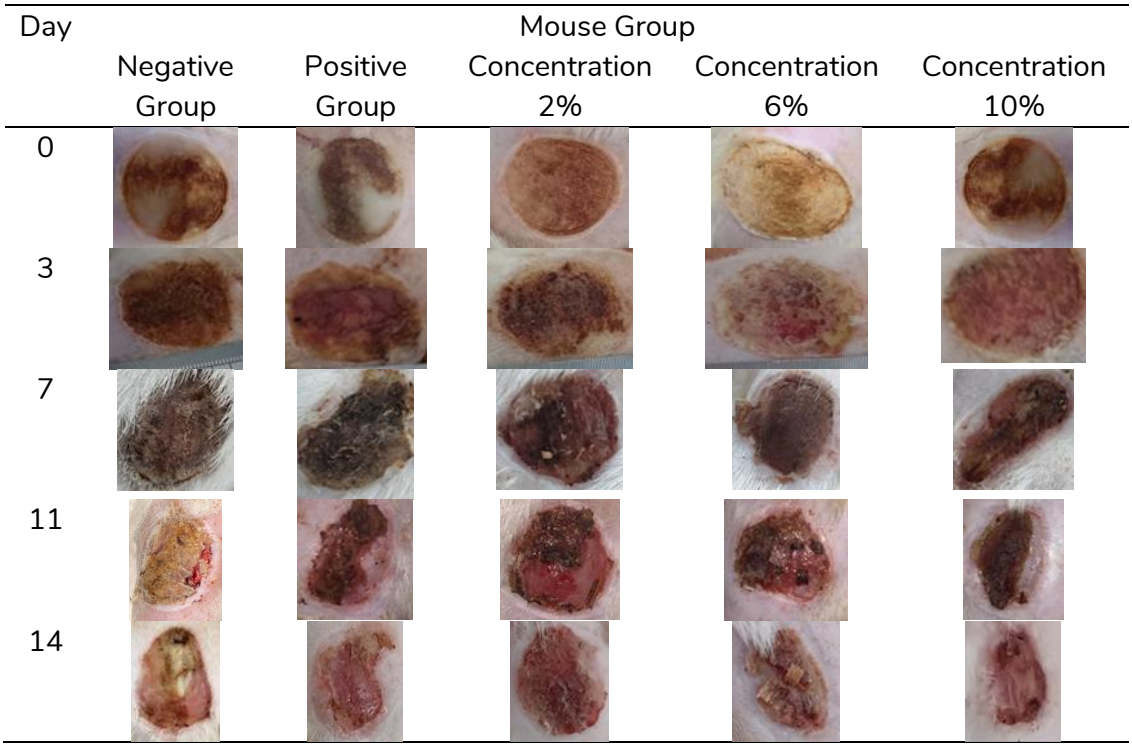

Figure 2. Wound Area Day 0-14.

Wound area ratio test observations were observed for 14 days where the negative group was given ointment base, the positive group was given bioplacenton, the 2% concentration group was given nutmeg leaf extract ointment from fak-fak with a concentration of 2%, the 6% concentration group was given nutmeg leaf extract ointment from fak-fak with a concentration of 6%, the 10% concentration group was given nutmeg leaf extract ointment from fak-fak with a concentration of 10%. The results of the difference in rat wound area showed that the positive group and the 2% concentration group had almost the same healing rate. While the 6% concentration group and the 10% concentration group have almost the same healing rate but the 10% concentration group has a faster healing rate among other groups. In contrast, the negative group has a much longer healing rate among other groups. This shows that the higher the concentration of nutmeg leaf extract from fak-fak (*Myristica argentea* Warb), the better the wound healing process in rats.

The results of the one way anova test that have been carried out can be seen in table 3 where the results obtained > 0.05 are 0.735 which means there are no significant results,

so it can be concluded that there is no significant difference between the treatment groups of fak-fak nutmeg leaf extract ointment.

CONCLUSION

Based on the research that has been done, it can be concluded that the ointment preparation of fak-fak nutmeg leaf extract can provide anti-inflammatory activity in wistar male white rats, and the most effective ointment preparation is an ointment preparation with an extract concentration of 10%, this shows that the higher the concentration of ointment extract of nutmeg leaves from fak-fak (*Myristica argentea* Warb) the better the healing process in wistar male white rats.

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