

Xylocarpus granatum Mangrove Fruit Extract and Sodium Alginate Extract Lotion as Potent Wound Treatment Medicine

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ABSTRACT

A preliminary study to gauge the antimicrobial potency of *Xylocarpus granatum* mangrove fruit extract and sodium alginate extract against pathogenic microbe from the species *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. Lotions made of mangrove fruit extract (Sample 1) lotion and of a mixture of fruit extract and sodium alginate extract lotion (Sample 2) were topically tested on incision cut wound on mice abdominal area. The lotion was applied daily for five consecutive days. Observation on the test subjects were conducted to determine the rate of blood agglutination, tissue recovery rate, and fibroblast development rate using histology. The results showed that *X. granatum* extract lotion displayed significant antimicrobial activity against both pathogenic microbe species and did not show any conflict with the microbial activity found in sodium alginate lotion. Lotion adhesiveness test measured sample 1 at 0.26 seconds and sample two at 0.16 seconds. Both samples were shown to be oily in water. Subject observation showed blood coagulation on the first day, onset of tissue recovery on the second day and by the third day the wound had undergone complete tissue recovery. Observation on the fifth day showed that fibroblast tissue on the subject with sample 2 treatment was more solid than that with sample 1 treatment. It was concluded that the mix of *X. granatum* mangrove fruit and sodium alginate extracts showed most potency in wound treatment.

Key words: *X. granatum*; incision wound; lotions; skin; histology; fibroblast.

INTRODUCTION

Mangrove is a plant species abundant in tropical coasts and can be found in many coastal areas in Indonesia. If one walks along the coast which is home to mangrove ecosystem there, one can see 2 types of specific mangrove roots; terrain and aerial roots. These specific root placements are what makes mangrove ecosystem, being rich in nutrition, important for thriving marine life as feeding ground and shelter.

Mangrove ecosystems are also beneficial to humans. They can reduce erosion and abrasion in coastal areas. In the past, people also use the trunk of mangrove trees for pyres. Today, advancement in science and technology also discovers that mangrove ecosystem can contribute to the economic betterment of the surrounding community. Dead leaves of mangrove plants are broken down by bacteria into nutrition. These decomposing bacteria species have the potential to be used in bioactivator products (Pringgenies *et al.*, 2016). On the other hand, mangrove wastes can also become materials for natural-dye batik (Pringgenies *et al.*, 2013; Pringgenies *et al.*, 2017a; Pringgenies *et al.*, 2018). The fruits of some mangrove species are turned into food products, such as crisps, syrup, etc (Pringgenies *et al.*,

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2017b). However, fruits of other mangrove species cannot be used as food due to its extremely bitter taste, such as *Xylocarpus granatum*. The local community in Central Java, Indonesia, refers to the fruit of *X. granatum* as “the bride’s fruit” for its long-known, skin-emolliating properties. Based on this reference, the fruit of *X. granatum* may have to potency as an ingredient in wound treatment lotion, in combination with other natural ingredients such as alginate sodium. This research aims to determine antimicrobial potency of *X. granatum* fruit and alginate sodium extract against prevalent dermal pathogenic bacteria namely *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. This study will specifically determine the potency of lotion preparation with *X. granatum* extract (Sample 1) and of lotion with *X. granatum* extract in combination with alginate sodium (Sample 2) for wound recovery treatment on the lab mice.

MATERIALS AND METHODS

The material used in this study is lotion preparation with alginate sodium extracted from *Sargassum* sp. seaweed and lotion preparation with mangrove extract from *X. granatum* species. This study used 1-month old mice (*M. musculus*) of BALB/C strain as live subjects. The live subjects were certified by LPPT Unit IV Universitas Gadjah Mada, Yogyakarta. This study was approved by Commission for Ethical Health Research (KEPK) School of Medicine Universitas Diponegoro and

dr. Kariadi Central General Hospital Semarang under Ethical Clearance No. 10/EC/H/FK-RSDK/III/2018.

Alginate Sodium and *X. granatum* Fruit Extraction

Prior to extraction process, fresh *Sargassum* sp. seaweed samples were rinsed with fresh water to reduce salt content, after which they were air-dried and cut into pieces of $\pm 0,5$ cm. The extraction process began by mixing 50 mM EDTA and Na_2CO_3 5% with additional HCl until the pH of the solution reached 8.4 from the original 11. The solution was used to soak *Sargassum* sp. samples for 24 hours, during which it was stirred using a magnetic stirrer. After that, the samples were sieved from the solution. The solution was then mixed with KCl 0.13 M and cold ethanol 96% on 1:1 volume ratio. The mixture was put into centrifuge on 3500 rpm for 5 minutes. The resulting pellets were then removed and put into oven at 60 °C for 24 hours until the alginate was completely dehydrated and ready to be pulverized (Yudiati & Isnansetyo, 2017).

Fresh samples of two *X. granatum* fruits, weighed at 2.650 kg, were collected and cleaned. Samples were dehydrated in an oven at 45 °C for 14 days. Dehydrated samples were then diced into 2x2 cm pieces and diluted in 1000 mL of n-hexane using an erlenmeyer flask with airtight cover of aluminum foil and incubated for 24 hours. After incubation, the pulp was removed from the solution. The pulp was then re-diluted with methanol solution for 2 hours. The methanol

Table 1. Formation of inhibition zones in antimicrobial test of *X. granatum* and alginate sodium extracts against *P. aeruginosa* and *S. epidermidis*.

| Concentration (ppm) | Alginate sodium extract inhibition zone (mm) | | <i>X. granatum</i> fruit extract inhibition zone (mm) | |
|---------------------|--|-----------------------|---|-----------------------|
| | <i>P. aeruginosa</i> | <i>S. epidermidis</i> | <i>P. aeruginosa</i> | <i>S. epidermidis</i> |
| 100 | - | - | - | 2.48 |
| 250 | - | - | - | 2.96 |
| 500 | - | - | - | 0.83 |
| 1000 | - | - | 1.11 | 1.23 |
| Control + | - | - | 2.03 | 15.31 |
| Control - | - | - | - | - |

dilution process was repeated until no changes in color occurred. The final dilution solvent was then evaporated using a rotary evaporator (Hu *et al.*, 2018) at 35-40 °C. The resulting extract was then put into a vial and weighted.

Antimicrobial Qualitative Test

Antimicrobial qualitative test was performed to determine the effect of alginate sodium extract and *X. granatum* fruit extract form inhibition zones against pathogenic bacteria, or in other words the potency of both extracts as antimicrobial agents. The test was performed by using 10 µL/disk of samples against. Each sample on 100, 250, 500, and 1000 ppm concentration was dispensed on paper disks with bacteria culture (Soares *et al.*, 2020). The control treatment was amoxicillin antibiotics at 1000 ppm concentration. Antimicrobial activity would be determined by the formation of inhibition zones at the periphery of the dispensed samples.

Making Lotion Preparations

Lotion preparations were made by mixing four parts; Part A, Part B, Part C and Part D. Part A was made by mixing NaEDTA and distilled water, mixed with a solution of boiling water and Carbopol. Part B was made by diluting propylene glycol, glycerin, nipagin, and nipasol in distilled water and heated at 65 °C until thoroughly mixed. Part A was then mixed with Part B. Part C was prepared by mixing Liquid paraffin, stearic acid, glycerol monostearate, cetyl alcohol and distilled water at 65 °C. The mixture of Part A and Part B was added into Part C, after which it was homogenized. Part D was made by diluting Triethanolamine (TEA) in distilled water, which was then added into the mixture of Part A, Part B and Part C. The final solution of all parts was then homogenized using a stamper and added with distilled water until the ideal cream base quality is achieved.

Lotion Preparation Quality Test

The lotion preparation quality test in this study includes organoleptic (texture, color, and odor) test, acidity test, viscosity test, spread rate

test, adhesiveness test, and lotion type test. Organoleptic test involves a series of test using human sensory (visual, olfactory, somatosensory) to measure viability of a product for human consumption (Olesiuk *et al.*, 2013). This study conducted test on the texture, color and odor of the alginate sodium extract and mangrove plus alginate sodium lotion preparation. Acidity of the lotion was measured using pH meter, and the viscosity test was performed using Brookfield viscometer. The spread rate of lotion in this study was determined by measuring the constant diameter of the lotion spread between 10 x 10 cm glass plates which was pressed by 50, 100, 150, 200, and 250 grams of weight for one minute each time. The lotion adhesiveness test was conducted using two glass objects pressed under a 50-gram weight.

Shaving, Incision, and Lotion Administration Treatment

Sixteen female mice were acclimatized for seven days prior to the subject processing. Hair from the abdominal part of each subject were removed and ± 0.5 cm incision was made using sterilized scalpel. After the incision, control, non-extract lotion and extract lotion treatment were administered to the respective subjects. After lotion treatment, observation conducted on blood coagulation and tissue reparation. The treatments were administered once a day in five consecutive days (Suwiti, 2010).

Histology

Histology preparation was made using methods in accordance to Luna (1968) and Cullin & Dun (1974). Dermal tissue sample from all the subjects were fixated in Buffer Neutral Formalin (BNF) 10% solution which would then be dehydrated with alcohol 70%. The samples were cleansed with formalin 10% I, formalin 10% II, and formalin 10% III each for one session respectively. The cleansed samples were then put into another dehydration process with alcohol 70%, alcohol 96%, absolute alcohol I, absolute alcohol II, and absolute alcohol III and soaked in xylol I, xylol II, xylol III, liquid paraffin I and liquid paraffin II. For

the final sample preparation, all samples were blocked with liquid paraffin until frozen, followed by cutting using microtome knife (Suwiti, 2010).

The prepared skin tissue sample were then stained with haematoxylin eosin (HE) using Harris eosin staining method. The samples were soaked three times in xylol with each session lasting for 5 minutes. The samples were then soaked with absolute alcohol three times for 5 minutes each session. Afterwards, the samples were soaked in distilled water twice for one minute each session. They were then soaked with acid alcohol 10% for 5 to 7 minutes. The samples were again soaked in distilled water for 1 and 15 minutes prior to haematoxylin soaking for 15 minutes. The samples were soaked in eosin afterwards. The stained samples were then soaked in alcohol 96%, followed by another soaking in absolute alcohol 2 times for 3 minutes each session. The last process involved soaking the samples twice in xylol, with 5 minutes duration for each session (Suwiti, 2010).

Histological Observation of Preparations

The observation in this study employed light microscopy using Leica microscope, of German make, with 40x10 and 100x10 magnification factor. Observed variables include histology of tissue reparation and identification of fibroblast as well as inflammatory cells (Suwiti, 2010).

RESULTS AND DISCUSSION

Antimicrobial Test Results of Alginate Sodium and *X. granatum* Fruit Extracts

The test results showed that alginate sodium extract did not display any antimicrobial activity against the pathogenic bacteria used in this study, *S. epidermidis* and *P. aeruginosa*. However, *X. granatum* fruit extract showed antimicrobial activity, of which the highest was found with 250 ppm concentration (2.96 mm) and the lowest with 500 ppm (0.83 mm) (Table 1).

Lotion Preparation Quality Test Results

From the observation of the results, there was no marked organoleptic distinction between

Table 2. Organoleptic test results of mangrove extract and alginate sodium extract lotion.

| Specification | Mangrove Extract Lotion | Mangrove plus Alginate Sodium Lotion |
|---------------|-------------------------|--------------------------------------|
| Texture | Semi-solid | Semi-solid |
| Color | Pale beige | Pale beige |
| Odor | Common of lotion | Common of lotion |

Table 3. Viscosity, adhesiveness, and acidity test results of mangrove extract and mangrove plus alginate sodium extract lotions.

| No. | Sample | Viscosity (Cp) | Time (Seconds) | pH |
|-----|--------------------------------------|----------------|----------------|------|
| 1. | Mangrove extract lotion | 6.203 | 0.2 | 6.18 |
| 2. | Mangrove plus alginate sodium lotion | 7.774 | 0.16 | 6.85 |

Table 4. Tissue reparation rate on live subjects.

| Lotion | Day 1 | Day 2 | Day 3 | Day 4 |
|---------------------------------------|-------|-------|-------|-------|
| Mangrove extract | × | √ | √ | √√ |
| Mangrove plus alginate sodium extract | × | √ | √√ | √√ |

lotions with mangrove extract and mangrove plus alginate sodium extract. Both products exhibited almost similar texture, odor and coloration, as shown in Table 2.

Viscosity Test Result of Mangrove Extract Lotion and Mangrove Plus Alginate Sodium Extract Lotions

The test produced marked difference in the viscosity between mangrove extract and mangrove extract plus alginate sodium lotion preparations. The mangrove plus alginate sodium preparation was found to have higher viscosity (7.774 Cp) compared to the mangrove extract

lotion preparation (6.203 Cp). The complete results are presented in Table 3.

Adhesiveness Test Results of Mangrove Extract and Mangrove Plus Alginate Sodium Lotion

Results of the test showed that mangrove lotion preparation have more adhesion time (0.26 seconds) in comparison with mangrove plus alginate sodium lotion preparation (0.16 seconds) (Table 3).

Acidity (pH) Test Results of Mangrove Extract and Mangrove Plus Alginate Sodium Extract Lotions

The test results showed that both lotions have no noticeable difference in acidity, mangrove plus alginate sodium extract lotion being of slightly higher acidity (6.85 pH) compared to mangrove extract lotion (6.18 pH) (Table 3).

Cream Type Test Results of Mangrove Extract and Mangrove Plus Alginate Sodium Extract Lotions

Microscopy observation of the samples found that both lotions are oil-in-water type. This finding

was deduced from results of emulsion type identification by adding water to the lotion, in which case did not disrupt the emulsion stability of the cream. The lotion samples were given methylene blue stain, which is highly soluble in water. Image B showed that the stain was diluted in water, which meant that water was the diluting phase.

Histology of Treatment Results

Light microscopy observation results of mangrove extract and mangrove plus alginate sodium extract on day 1 until day 4 consecutively showed no blood agglutination. Subjects with mangrove plus alginate sodium extract treatment showed complete tissue reparation on day 3, whereas subjects with mangrove extract treatment showed complete tissue reparation on day 4. The complete results of light microscopy analysis is presented in Table 4.

The results of fibroblast formation observation using micrography showed that subjects with mangrove lotion treatment had better fibroblast formation rate than subjects with mangrove plus alginate sodium treatment as seen in comparison

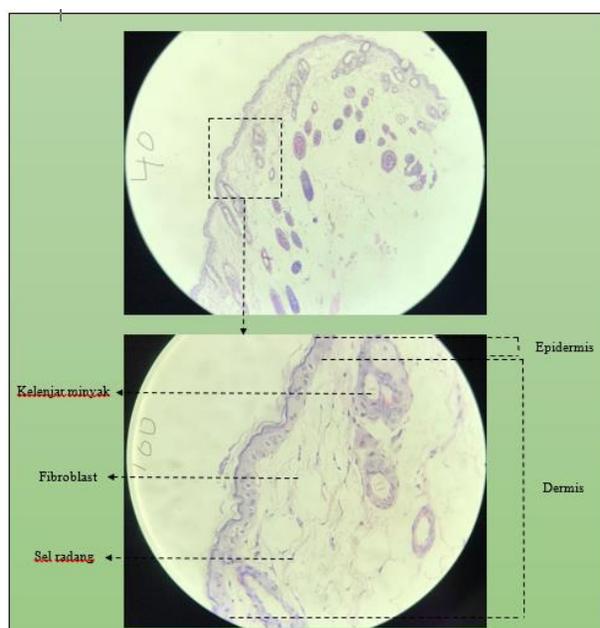


Figure 1. Micrograph of dermal tissue of subjects with mangrove plus alginate sodium extract treatment.

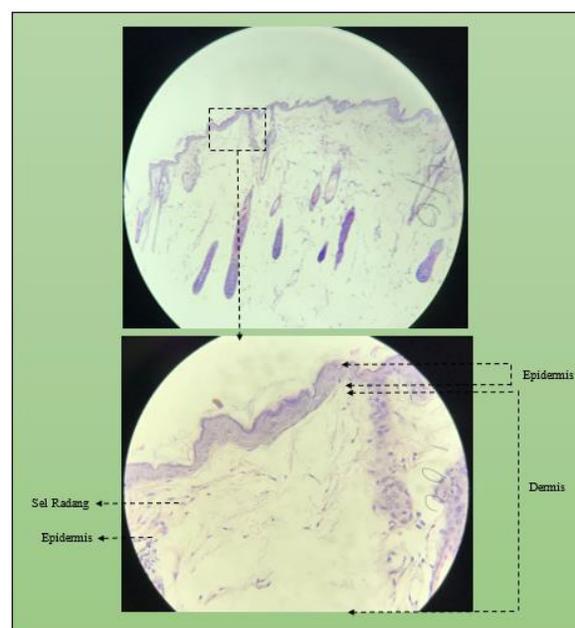


Figure 2. Micrograph of dermal tissue of subjects with mangrove extract treatment.

between image 1 and image 2.

Discussion

The absence of antimicrobial activity in alginate sodium extract against *S. epidermidis* and *P. aeruginosa* showed that the extract does not contain any antimicrobial substances. Indeed, the main use of alginate sodium has been as an adhesive agent. On the other hand, *X. granatum* fruit extract showed antimicrobial activity against the pathogenic test bacteria in this study, which confirmed the local wisdom of its medicinal properties. In Thailand, the fruit of this particular species is widely used to treat diarrhea (Das *et al.*, 2015). Diarrhea is a disease known to be caused by *E. coli* pathogenic bacteria. This study confirms *X. granatum* fruit extract antimicrobial properties against *S. epidermidis* and *P. aeruginosa*. *S. epidermidis* is a species which thrives in waste water (Pringgenies *et al.*, 2018) which is a potential pathogenic bacteria in wounds. *P. aeruginosa* is a species which is commonly found in wounds.

The viscosity test results of both lotions found that mangrove plus alginate sodium extract lotion to be more viscous (7.774 Cp) compared to mangrove extract lotion (6.203 Cp). It was postulated that the presence of contributes to more viscosity of the lotion, due to the fact that alginate had been used widely as thickener (Parreidt *et al.*, 2018). The adhesiveness test result analysis found that mangrove plus alginate sodium extract lotion has lower adhesiveness time than mangrove extract lotion. This finding demonstrated that mangrove extract possesses high adhesiveness on its own. Lower acidity (6.18 pH) of mangrove extract lotion contributes to the better adhesiveness compared to the mangrove plus alginate sodium lotion (6.85 pH).

Histology analysis of dermal tissue from subject using light microscopy found that mangrove plus alginate sodium extract treatment subjects had undergone complete tissue reparation by day 3 after incision was made. However, similar level of tissue reparation in subjects with mangrove extract treatment was found only in day 4. Histology observation on fibroblast formation showed that subjects with mangrove plus alginate

sodium extract treatment exhibit better fibroblast formation rate than subjects on mangrove lotion treatment. The formation of fibroblast is one of the indicators of good tissue reparation. Fibroblast is formed during the proliferation phase (Wosgrau *et al.*, 2015), which is a key element in the formation of collagen, an important building block of dermal tissue (Wasitaatmadja, 2007). The rapid recovery of subjects with mangrove plus alginate sodium treatment owes to the presence of alginate, which expedite tissue reparation process, and *X. granatum* fruit extract, which has been found in this study to exhibit antimicrobial properties.

CONSLUSSION

This study concludes that rhe extract of *Xylocarpus granatum* fruit possesses antimicrobial potency against *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* pathogenic bacteria and lotion with *X. granatum* fruit extract on its own and in combination with alginate sodium extract aids dermal tissue reparation and wound recovery process. Lotion with *X. granatum* fruit plus alginate sodium is found to be the better treatment for wound recovery with more immediate results.

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