

ANTI-INFLAMMATORY ACTIVITY OF PHARMACEUTICAL GEL OF ETHANOLIC EXTRACT FROM MARINE SPONGE *Xestospongia Sp*

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Abstract

The aim of this study was to determine the characteristics of the gel formula based on its organoleptic properties, pH, viscosity, dispersion and homogeneity by stability test carried out using the cycling test method and to determine the anti-inflammatory activity of the *Xestospongia Sp.* in male white mice (*Mus musculus*) by creating an artificial edema on the mice left foot induced by 1% λ -carrageenan. The gel formula from the ethanol extract of *Xestospongia Sp.* sponge was physically stable in terms of its organoleptic observation, homogeneity, pH and viscosity test. However, the results that were obtained after dispersion test did not fulfill the requirements. In this study, the gel formula of the ethanol extract of *Xestospongia Sp.* sponge was administered on the mice left foot by using the variations in extract concentration of 0.02%, 0.03%, and 0.04%, and the gel without extract as a negative control and Galtaren®gel (1% Diclofenac Sodium) as a positive control. The evaluated data were in the form of mice leg edema volume measured based on its percent of inflammation and percent of inflammatory inhibition and observed for 360 minutes. The data were analyzed by using the Kruskal-Wallis test followed by the Mann-Whitney test with a confidence level of 95%. The results of this study showed that the gel formula of ethanol extract of *Xestospongia Sp.* sponge has an anti-inflammatory effect on each concentration, and the formula that has a large anti-inflammatory effect was obtained at extract concentration of 0.04%.



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INTRODUCTION

Indonesia is the largest archipelago country in the world and has a beach length of 81,000 km which is rich in coral reefs and other marine biota. Since the 1980s, the attention of the world of medicine has been directed towards marine biota as a potential resource. Some marine biota that are known to produce active compounds include sponges, molluscs, Bryozoa, tunicata and others (Cribb & Ford, 2009). One of the marine biota that is currently being studied is sponges. Sponges are one of the components of the composition of coral reefs that have the potential of bioactive compounds that have not been widely used (Mehub et al., 2014).

The sponge consists of three main classes, namely Calcarea, Demospongiae, and Hexactinellidae. The Demospongiae class is a sponge group that dominates

more than 90% of sponge species (Marzuki, 2018). One species of this class that can be used as a medicinal ingredient is *Xestospongia Sp.* which is known to have various activities such as anticancer (Murniasih, 2003), antiplasmodial, antibiotic, antifungal, cardiotoxic, cytotoxic, and antimalarial (Sadarun et al., 2018).

The study conducted by El-Shytany et al (2015) reported that methanol extract from *Xestospongia* sponge showed an anti-inflammatory effect that is thought to originate from the content of sterol compounds on sponges. Based on research conducted by Amri (2018), it was shown that the ethanol extract of *Xestospongia sp.* has anti-inflammatory ability which was measured using the red blood cell stabilization method with the stability percentage of the ethanol extract of *Xestospongia Sp.* at 50 ppm (56%), 100 ppm (67%), 200 ppm (69%), 400 ppm (76%), 800 ppm (83%), 1600 ppm (87%) and 3200 ppm (88%) and categorized

as toxic with an LC_{50} value of 440.53 ppm for *Artemia salina* Leach.

Inflammation is the body's attempt to inactivate or damage organisms that attack, eliminate and regulate the degree of tissue repair (Wijaya *et al.*, 2015). The inflammatory response is characterized by conditions such as redness, heat, pain, swelling and dysfunction (Sukmawati, 2015). Anti-inflammatory drugs commonly used are divided into two, namely steroid anti-inflammatory and non-steroidal anti-inflammatory drugs (NSAIDs) (Widiyantoro *et al.*, 2012). Both of these groups can relieve inflammatory reactions well but long-term use can have side effects (Hardman & Limbird, 2003). Based on this, there is a lot of development of anti-inflammatory products derived from natural ingredients and replacing routes through topical drug administration.

One of the topical dosage forms is gel. Gel is a semisolid system made of small inorganic particles or large organic molecules penetrated by a liquid. Gel has the ability to spread well on the skin, easily dries, forms a film that is easy to wash and gives a cool feeling to the skin (Sayuti, 2015).

Based on the description above, the authors conducted a study to make anti-inflammatory gel preparations from *Xestospongia Sp.* Organoleptic observations will include the shape, color, and smell of the preparation during a certain storage time, followed by testing homogeneity, pH, dispersion, and viscosity of the preparation, and then testing the anti-inflammatory effects of gel preparations made on white male mice.

MATERIAL AND METHODS

Tools and Materials

The tools used in this study include beaker (Pyrex®), measuring cups (Pyrex®), hot plates (Stuart®), flask (Pyrex®), ovens (Froilabo ®), magnetic stirrer (Stuart®), pH meter, measuring pipette (Pyrex®),

modified pletismometer, rotary vacuum evaporator (Rotavapor, Buchi®), analytical scales (Precisa XB 220A®), and viscometer (Rion Rotor Viscotester VT-04®).

The materials used in this study include *Xestospongia Sp.* obtained from Soropia sea waters, Konawe Regency, Southeast Sulawesi, purified water, Carbopol 940 (Intraco), 96% Ethanol (technical), Galtaren® gel (1% Diclofenac Sodium), Green tea oil, Filter paper, Methyl paraben, 0.9% NaCl, Propylene glycol, Triethanolamine (TEA), and λ -Carrageenan 1%.

Methods

Extraction

Sample Sponge *Xestospongia Sp.* obtained from the sea of Soropia, Konawe Regency, Southeast Sulawesi. Extraction was done by maceration using 96% solvent. Maserate obtained was then concentrated with a rotary vacuum evaporator at a maximum temperature of 60°C until a thick extract was obtained.

Gel Formulation

Gel formula for the ethanol extract of *Xestospongia sp.* made as much as 100 g with variations in extract concentrations of 0.02%, 0.03% and 0.04%. The formula design can be seen in Table I.

Table I. Gel formulation

Materials	Function	Formula % (b/b)			
		F0	FI	FII	FIII
Extract of <i>Xestospongia sp.</i>	Active compound	-	0,02	0,03	0,04
Carbopol 940	Gel base	1	1	1	1
Triethanolamine	Stabilizer	0,5	0,5	0,5	0,5
Propylene glycol	Humectant	10	10	10	10
Methyl paraben	Preservative	0,2	0,2	0,2	0,2
Green tea oil	Flavor	0,05	0,05	0,05	0,05
Purified water	Carrier/solvent	Ad 100	Ad 100	Ad 100	Ad 100

Preparation of *Xestospongia Sp.* ethanol extract gel. starting with preparing tools and materials then each ingredient is weighed according to the calculations stated in the formula design. Carbopol is dispersed in warm water using a magnetic stirrer until it is homogeneous above the water bath at a temperature

of 70-80°C. Next to neutralize carbopol, add triethanolamine and stir again until a clear gel period is formed. After that, a number of extracts and methyl paraben were added which had been dissolved with propylene glycol and homogenized. Green tea oil is included when the mixture has been homogeneous according to the specified concentration.

Gel Stability Test

Stability evaluation is carried out by the cycling test method. The gel is stored at 4°C for 24 hours then released and placed at 40°C for 24 hours, this treatment is one cycle and carried out in six cycles. The parameters used were organoleptic test, pH test, viscosity test, dispersion test and homogeneity test to determine the stability of gel preparations both before the cycling test and after the cycling test.

Organoleptic Gel Test

Organoleptic observations can be assessed from the texture of the preparation including changes in color and gel odor. Observations were made on the newly created and stored gel (Sharon *et al.*, 2013). A stable gel must show the same character in the same color and smell before and after accelerated storage conditions (Pakki *et al.*, 2009).

PH Gel Test

PH testing was carried out using a pH meter by means of a gel preparation weighed 1 g then dissolved with 10 mL of distilled water and stirred until evenly distributed. After that the pH meter is dipped into the solution and the results are recorded (Saraung *et al.*, 2018).

Gel Viscosity Test

The viscosity of the formulation is determined by Rion Rotor Viscotester VT-04 using spindle no. 3. The gel is inserted in the cup glass, then the spindle is dipped in the gel not to touch the container. Then the viscometer was turned on and it was seen what scale was designated (Aswal *et al.*, 2013). A good semisolid preparation has a viscosity value of 50 dPa.s to 400 dPa.s (Garg *et al.*, 2002).

Gel Spread Test

Spread test is done to ensure even distribution of gel when applied to the skin which is done as soon as the gel is made. The gel is weighed as much as 0.5 g then placed in the middle of a scale round glass. On top of the gel is put another round glass or other transparent material and ballast so that the weight of round glass and ballast 150 g, let stand a minute, then note the spread diameter. Good gel spread between 5-7 cm (Mappa *et al.*, 2013).

Gel Homogeneity Test

Homogeneity tests were carried out to see the homogeneity of the preparations made. The test is done by applying a gel on a piece of glass or other transparent material that is suitable, the preparation must show a homogeneous arrangement and no coarse grain is seen (Ministry of Health of the Republic of Indonesia, 1995).

Grouping and Acclimatization of Test Animals

Test animals were grouped into five groups where each group consisted of five test animals. Thirty healthy mice (20-30 g) were selected for this study. Mice were fed and drunk then acclimatized for a week before conducting the experiment.

Making Inflammation Indicators

A total of 0.1 g carrageenan in the form of 1% carrageenan solution made by weighing and then put in a 10 mL volumetric flask and then filled with a physiological solution of sodium chloride (NaCl) 0.9%, to the marking line. Then incubate at 37°C for 24 hours (Sujono *et al.*, 2012).

In Vivo Antiinflammatory Test on Male White Mice

White mice (*Mus musculus*) were grouped into five groups consisting of the positive control (diclofenac sodium gel), negative controls (gel without active ingredients), and the test group (three concentrations of gel extract of *Xestospongia* sp.) Ethanol extract.

Mice were fasted for \pm 18 hours before testing and drinking water was still given. On the day of testing, each animal is weighed and marked on its left ankle, then the volume of the left leg of the mice is measured by dipping the leg of the mouse into the pletismometer to the mark boundary and recording the initial volume (V_0), which describe the volume of the foot before being treated. Then each mouse leg is subplanted with 0.1 mL of 1% carrageenan suspension. One hour after injecting carrageenan suspension, each group was treated topically according to the group. After 60 minutes of giving an anti-inflammatory gel, the volume of the left leg of the mouse is measured again with a pletismometer. Changes in the level of swelling that occurs is recorded as the volume of the left foot of the mouse (V_t). Measurements are carried out every 60 minutes for 360 minutes.

Data Analysis

Percentage of inflammation can be calculated by the formula below (Kosala *et al.*, 2018):

$$\text{Percent inflammation} = (V_t - V_0) / V_0 \times 100\%$$

Where :

V_t = Inflammation volume after time t

V_0 = Initial volume of mice feet

Percent inhibition of inflammation is calculated by the formula below:

$$\text{Percent inflammation inhibition} = (a - b) / a \times 100\%$$

Where :

a = Percent of inflammation in the average negative control group

b = Percentage of inflammation of the average group treated with the test material or comparison drug.

RESULTS AND DISCUSSION

Extraction

Xestospongia Sp. which was used was obtained from the sea waters of Soropia, Konawe Regency, Southeast Sulawesi. *Xestospongia Sp.* which is used as much as 5.3

kg. The results of the maceration process using 96% ethanol solvent, obtained as much as 205.28 g thick extract with the yield of 3.87%.

Organoleptic Gel Test

Observations of organoleptic preparations for *Xestospongia sp.* Gel in Table 2 can be seen that the concentration gel formula 0%, 0.02%, 0.03%, and 0.04% did not show changes before and after the cycling test. Organoleptic test gel dosage forms both before and after cycling test on all formulas showed the form of semisolid gel and the absence of phase separation, this was due to the active substance and gel base homogeneously and the amount of gelling agent sufficient to stabilize the gel (Wathoni *et al.*, 2009). The organoleptic test for color showed the intensity of the color of the gel preparation which increased with increasing concentration of the extract on the preparation. The color test results before and after the cycling test showed stable results which were marked by no change in color on all formulas. Organoleptic tests for odor before and after cycling test showed stable results with a distinctive odor from green tea flavoring.

Table 2. Observations of organoleptic gel preparations

Concentration of Extract (%)	Observation	Form	Color	Odor
0	Before cycling	Semisolid gel	White (clear)	Typical greentea
	After cycling	Semisolid gel	White (clear)	Typical greentea
0,02	Before cycling	Semisolid gel	Rather yellow	Typical greentea
	After cycling	Semisolid gel	Rather yellow	Typical greentea
0,03	Before cycling	Semisolid gel	Rather yellow	Typical greentea
	After cycling	Semisolid gel	Rather yellow	Typical greentea
0,04	Before cycling	Semisolid gel	Rather yellow	Typical greentea
	After cycling	Semisolid gel	Rather yellow	Typical greentea

PH Gel Test

As shown in Figure 1, after the cycling test there was an increase in pH. This is due to one of the additives in the gel preparation which is propylene glycol.

According to Rowe *et al* (2009), propylene glycol at low temperatures will remain stable, but at high temperatures, propylenglycol will be oxidized to propionaldehyde, lactic acid, pyruvic acid, and acetic acid. The oxidation process of propylene glycol can cause an increase in pH of the gel preparation. However, the range of pH values before and after cycling test is still within safe limits for topical preparations.

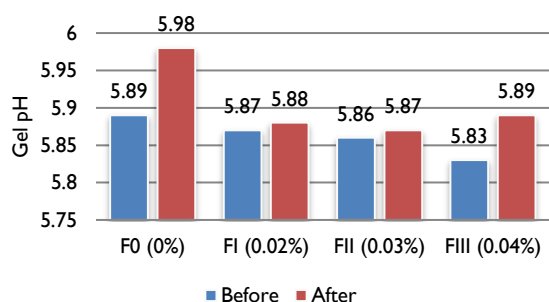


Figure 1. PH gel before and after cycling test

Gel Viscosity Test

The gel viscosity test results as shown is Figure 2 indicated that after cycling test there was an increase in viscosity. This could be due to a case hardening event that would make the preparation surface dry, due to the evaporation of water on the preparation. If the relative humidity of the air outside is lower than the relative humidity of the material, then the material will vaporize the water it contains. High water concentrations in gel preparations allow desorption, where water in the gel will evaporate which causes the gel to become denser so that the viscosity increases.

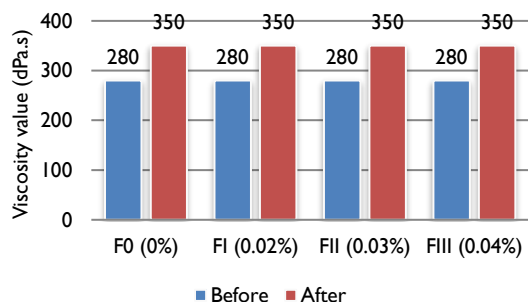


Figure 2. Gel viscosity before and after cycling test

Gel Spread Test

The results of the scattered power test results as shown in Figure 3 show that the spread of the gel will be greater with the increase in the amount of concentration of the extract added. But there was a decrease in the spread of gel after cycling test, this was in accordance with the increase in gel viscosity. The viscosity value is inversely proportional to the spread value of a gel preparation, so that if the gel has a high viscosity value, the value of the spreadability of the gel will be low. Based on this, all gel preparations did not meet the requirements for the semi-solid stock spread parameters of 5-7 cm (Garg *et al.*, 2002).

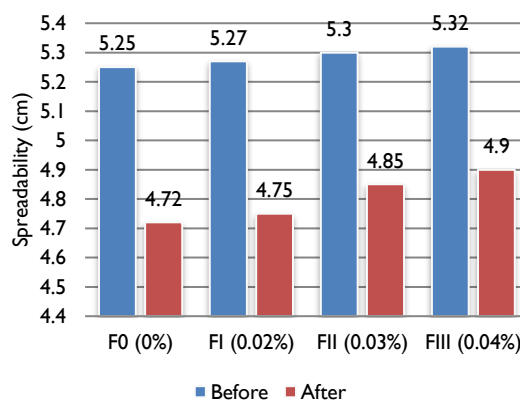


Figure 3. Gel spreadability before and after cycling test

Gel Homogeneity Test

The results of the observations on the gel homogeneity test of *Xestospongia* Sp. showed that the gel with extract concentrations of 0%, 0.02%, 0.03%, and 0.04% showed no lumps or coarse grains as can be seen in Table 3. This shows that the preparations made have a homogeneous arrangement, both before and after the cycling test.

Table 3. The observation of gel homogeneity

Concentration of Extract (%)	Homogeneity	
	Before cycling test	After cycling test
0	Homogeneous, no coarse grain	Homogeneous, no coarse grain
0.02	Homogeneous, no coarse grain	Homogeneous, no coarse grain
0.03	Homogeneous, no coarse grain	Homogeneous, no coarse grain
0.04	Homogeneous, no coarse grain	Homogeneous, no coarse grain

In Vivo Antiinflammatory Test on Male White Mice

Antiinflammation is the term for agents / drugs that work against or suppress the inflammatory process. The method of testing the anti-inflammatory effects of a drug candidate based on the ability of the test drug reduces or suppresses the degree of uedema in experimental animals. The gel formula which has been tested for stability is then tested for anti-inflammatory effects on white male mice test animals. Male sex is chosen so that the inflammatory response is not influenced by the estrogen hormone (Sativa *et al.*, 2014). Before testing, the test animals were acclimatized for a week with the aim of giving the animals time to adapt to the new environment. Furthermore, each mouse that will be used, fasted for approximately 18 hours and only given a drink, this is intended to avoid the possibility of the influence of food on the effect of the gel to be tested so that the gel given can provide a good anti-inflammatory effect.

The testing of antiinflammatory activity in this study was by inducing λ -carrageenan 1% on the left foot of the mice. Based on the research that has been done that after carrageenan administration, the legs of mice are inflamed. Carrageenan induction results in the formation of inflammation consisting of two phases, 1-2 hours after carrageenan injection, causing trauma due to inflammation caused by carrageenan. In the first phase there is the release of serotonin and histamine to the site of inflammation and an increase in prostaglandin synthesis in damaged tissue. In the second phase prostaglandin release occurs and is mediated by bradykinin and leukotriene (Manurung & Sumiwi, 2016). Inflammation that occurs in the legs of mice is seen with the onset of swelling and redness which is a feature of inflammation. This can be seen by increasing the volume of mice feet measured by a modified pletismometer. This method with the pletismometer tool was chosen because it has advantages in terms of implementation that is easier and simpler (Duarte *et al.*, 2012).

In this study the effectiveness of mice was carried out by comparing *Xestospongia Sp.* gels with preparations of diclofenac sodium gel (Galtaren®) as a positive control and gel without extract as a negative control. Gel formulas with various extract concentrations were 0.02%, 0.03%, and 0.04% used as test drug ingredients.

The analysis was performed on the results of changes in the volume of mice feet starting 60 minutes to 360 minutes or for six hours after carrageenan injection subplantar. From changes in the volume of mice feet can be calculated percent inflammation and percent inhibition of inflammation of mice. The results of percent inflammation in each treatment group and control group can be seen in Figure 4.

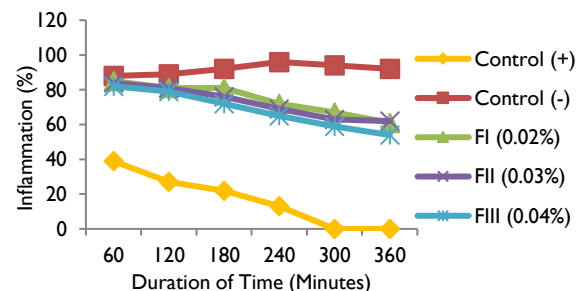


Figure 4. The average percentage of inflammation in the legs of mice

Figure 4 shows that there was a different decrease in inflammation in each treatment group and the control group. Giving the FI group gel (0.02%) experienced a decrease in inflammation in the 240th minute. Whereas in the FII group (0.03%) and FIII (0.04%) experienced the same decrease in inflammation starting in the 180th minute. In contrast to positive controls where the decrease in inflammation was greater than the test group, it occurred in the 60th minute. The graph data above shows that the FI group (0.02%), FII (0.03%), and FIII (0.04) takes longer than 360 minutes to cure inflammation. The percent value of the inflammatory barrier for each measurement time can be seen in Figure 5.

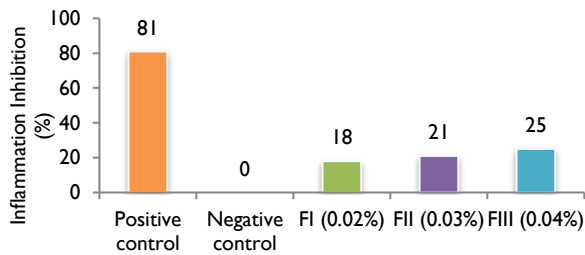


Figure 5. The average percentage of inhibition of inflammation of the legs of mice

Figure 5 shows that all treatment groups experienced a decrease in edema except negative controls that did not contain extracts. This is due to negative carrageenan-induced controls only given base gel treatment so that there is no healing stimulation to reduce edema and the edema response relies solely on the natural immune system of mice. This causes the edema to continue to enlarge, so the percentage reduction in edema is 0%.

Based on the Kruskal Wallis test on mice edema volume, the gel showed that the extract of the ethanol extract *Xestospongia Sp.* there were significant differences between the negative control group with positive control, FI (0.02%), FII (0.03%), and FIII (0.04%) with a significant value ($P < 0.05$) at the 60th minute to 360 minutes, so it can be concluded that these four groups have anti-inflammatory activity in inhibiting edema in the feet of mice compared to negative controls. It needs to be continued with the Mann Withney U test to find out whether there are significant differences between categories.

Based on the average inflammatory rate, the FI group (0.02%), FII (0.03%), and FIII (0.04%) had a greater average inflammatory value with a lower inhibition compared to the control positive. In the statistical test FI (0.02%), FII (0.03%), and FIII (0.04%) differed significantly from positive controls with significant values ($P < 0.05$). This shows that FI (0.02%), FII (0.03%), and FIII (0.04%) have lower antiinflammatory activity than positive controls positive control. Statistical tests on all formulas showed FI (0.02%), FII (0.03%), and FIII

(0.04%) there were no significant differences with significant values ($P > 0.05$), so that all formula has the same anti-inflammatory activity in inhibiting edema.

The anti-inflammatory effect is thought to be due to the activity of chemical compounds contained in *Xestospongia Sp.* consisting of flavonoids and sterols which are known as steroid alcohols, which are a subgroup of steroids. Steroid compounds have anti-inflammatory activity by the mechanism of activating glucocorticoid receptors by increasing or decreasing the transcription process of genes involved in the inflammatory process (Luliana *et al.*, 2017). Metabolic compounds that can inhibit inflammation are flavonoids. Flavonoids can inhibit the cyclooxygenase enzyme that plays a role in prostaglandin biosynthesis (Sativa *et al.*, 2014) and inhibits the lipookigenase enzyme that plays a role in leukotriene biosynthesis in inflammation formation (Sukmawati *et al.*, 2015).

CONCLUSION

Based on the results of the research that has been obtained it can be concluded that the ethanol extract of *Xestospongia Sp.* can be formulated in gel preparations with concentrations of 0.02%, 0.03% and 0.04%. The gel preparation formula is physically stable in terms of organoleptic observation, homogeneity test, pH and viscosity test. However, the results obtained in scattered power testing do not meet the requirements. Gel preparation from the ethanol extract of *Xestospongia Sp.* has an anti-inflammatory effect on each concentration and the formula which has a large anti-inflammatory effect is obtained at the extract concentration of 0.04%.

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