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Anti-Inflamatory Activity Evaluation of Sungkai Leaf Ethanol Extract (*Peronema Canescens* Jack) Using the Red Blood Cell Membrane Stabilization Method

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Abstract

Research has been carried out on the anti-inflammatory activity test of the ethanol extract of Sungkai leaf (*Peronema canescens* Jack) with the red blood cell membrane stabilization method. Purpose of study was to determine the anti-inflammatory activity the effect of the concentration of ethanol extract of sungkai leaves. Anti-inflammatory activity can be determined by measuring stability red blood cells, looking at absorbance of decomposition of hemoglobin so that the percentage of stability is obtained. Variations in the concentration of the test solution made were 4 variations in concentration, namely 15, 30, 60 and 120 ppm. The extract's ability to reduce inflammation. was then compared standard ibuprofen. Based on the study's findings, the percentage of erythrocyte membrane stability of sungkai leaf extract sequentially with concentrations of 15, 30, 60 and 120 ppm was 2.24%, 32.61%, 37.72%, 44.31%, while the comparison (ibuprofen) The percentage of erythrocyte membrane stability, respectively, with concentrations of 15, 30, 60 and 120 ppm were 24.1%, 36.2%, 49.4%, 63.4% and control 0.98%. The data from study were analyzed using one-way ANOVA which was followed by Duncan test. From this research is isconcluded The activity that reduces inflammation of the ethanolic extract of the sungkai leaf variation of the concentration regarding ethanolic extract of sungkai leaf *(Peronema canescens* Jack) gave an influence on the anti-inflammatory activity with an increase in the proportion of the stability of the red blood cell membrane following The rise in the concentration of the ethanol extractf the sungkai leaf.

Keywords: anti-inflammatory, *Peronema canescens* Jack, Stabilization of red blood cell membranes

Introduction

Inflammation is defined as a local reaction of tissues to infection or injury and involves more mediators than immune responses. Inflammation is the body's natural reaction to different

stimuli, such as infections and tissue injuries (Baratawidjaja and Rengganis, 2014). The existence An inflammatory process is distinguished by specific characteristics, including the appearance of redness and swelling in the affected area, heat, and the onset of pain. Cell damage due to inflammation occurs in the cThe cell membrane releases lysosomal enzymes from leukocytes. Arachidonic acid is then freed from precursor compounds and the synthesis of various eucosonoids occurs. Arachidonate metabolism produces prostaglandins that have various effects on blood vessels, nerve endings, and on cells involved in inflammation (Katzung *et al*., 2014). Therefore, drugs are needed that can inhibit the inflammatory activity.

In general, the treatment used to treat inflammation is steroid anti-inflammatory drugs (Steroidal Anti-inflammatory Drugs / SAID) and non-steroids (Nonsteroidal Anti-inflammatory Drugs / NSAID) which act as analgesics, antipyretics and anti-inflammatory. But anti-inflammatory drugs that do not contain steroids have aftereffects related inhibition of prostaglandin synthesis, especially occurring in the stomach, intestines, kidneys and platelet function. As for steroid drugs, some of the side effects that can be caused are electrolyte fluid disorders, hyperglycemia and decreased immune system (Tjay and Rahardja, 2010).

According to According to the WHO, approximately 80% of the global population continues to depend on traditional medicine, including the use of natural remedies plant-derived medicines because of the advantages of plants. Therefore, plants are more commonly chosen as holistic and organic remedies a the Treatment for various diseases, though scientific evidence remains insufficient truth about the efficacy of these plants. One of the plants that serves as a natural remedy for inflammation is the Sungkai leaf (Peronema canescens Jack). There has not been much research done on sungkai leaves, because research on this leaf as a medicinal ingredient in the community is still lacking, so It is essential to carry out basic testing To establish bioactivity this plant. According to the study findings, leaves of Sungkai (Peronema canescens Jack) contain a type of active compound Peronemin which functions as an anti-malarial drug (Andriani Fenny, et al., 2017). According to Ningsih et al. (2013), the results of the isolation of n-Hexane from Sungkai leaves (Peronema canescens Jack) obtained one compound, namely isolate B1, based on chemical reagent data that isolate B1 was positive for the terpenoid compound group and had anti-bacterial activity. Young sungkai leaves also contain flavonoids, which play a major role as red, blue and purple pigments found in most higher plants (Winkel-Shirley, 2001). Flavonoids have an antipyretic effect, as research results from Owoyele (2008) stated that the active ingredients of sungkai leaf extract (Peronema canescens Jack) which have analgesic, anti-inflammatory and antipyretic activity are flavonoids.

The young Foliage of the sungkai plant are used cure for colds, fevers, worms, used as a bath for women after childbirth, As an oral rinse to help prevent tooth pain, fever medication, pounded and pasted bruises, fever reduction, malaria and maintaining health (Ariefa Primair, et al. 2015).

As described earlier, the researcher intends conduct a study Concerning the anti-inflammatory activity test of sungkai leaf ethanol extract (*Peronema canescens* Jack) in *vitro*. The method used in this inquiry is Red blood cell membrane preservation technique, using goat blood cells whose lysosomal membrane is analogous to human blood cells. So that the stability

of the lysosomal membrane can be used as an illustration in testing the anti-inflammatory effect of a test material.

Research Method

Tool

Scales, blenders, erlenmeyer (Pyrex ®), beaker glass (Pyrex ®), test tubes (Pyrex), measuring cups (Pyrex®®), pH meters, flasks, droppipettes, mouthpieces, steam cups, cruising pliers, spatula, stirring rods, watch glass, maceration bottles, porcelain cruising, vacuum tubes, conventional columns (Pyrex®), ovens (Panasonic), *rotary evaporators* (IKA RV 10 Basic), UV-Vis spectrophotometer (T70), autoclave (Daihan labtech, Korea), centrifuge (Hettich EBA 20, Germany), desiccant, waterbath.

Material

The ingredients used are Sungkai Plant Leaves (*Peronema canescens* Jack), 70% ethanol, 95% ethanol, ammonia, filter paper, cotton, sodium chloride (NaCl), aquadesh, Mg powder, chloroform, FeCl3, concentrated sulfuric acid, concentrated hydrochloric acid, norites, Lieberman Burchad reagent, ammonia chloroform, mayer reagent, ibuprofen, goat red blood cells, sodium dihydrogen phosphate ($_{NaH2PO4}$. $_{H2O}$), disodium hydrogen phosphate (Na2HPO4.2H2O).

Sampling

The sample utilized consisted of Sungkai leaves. (*Peronema canescens* Jack) Included in the banja Loweh Godang jorong area, Bukit Barisan District, Lima Puluh Kota, West Sumatra

Sample Identification

The identification of samples took place at the Herbarium of the Biology Department,, Andalas University.

Making Sungkai Leaf Ethanol Extract

Fresh sungkai leaves are taken 5 kg and then cleaned and weighed, then dried by air in a place protected from direct sunlight. Making sungkai leaf extract by maceration. Sungka leaves (*Peronema canensces* Jack *)* are extracted by maceration method using a 70% alcohol solvent. The maceration results are separated by filtering, then the filtrate is concentrated by evaporation using a set of soclet tools so that a thick extract of young sungkai leaves is obtained. After that, this leaf extract is cooled and ready to be used or stored (Harborne, 1996).

Characterization of Sungkai Leaf Ethanol Extract

1. **Organoleptic Examination**

   It is performed through sensory observation, considering aspects such as hue, form, aroma, and flavor.

2. **Solubility Check**

The solubility check was executed out by dissolving viscous Infuse in water 95% ethanol (Djamal, 2010).

3. Determination of Extract Yield

Yield is a comparison between the extracted obtained and the initial simplicia.

% Yield =(Weight of extract obtained)/(Sample Weight) x 100

4. Determination of Drying Shrinkage

Put the evaporation dish that has been dried for 30 minutes in the oven at 105 °C, weigh 1-2 grams of extract, put it in the evaporation dish then weigh it again, then slowly shake the cup so that the extract is evenly distributed. Put it back in the oven. The steamer cup containing the extract is heated in 105°C for 1 hour. After that, take it out and cool in a desiccant and then weigh it. Do the same repetition as above until a constant weight is obtained.

% drying shrinkage =((B-A)-(C-A))/((B-A)) x 100%

Information:

A= Weight of empty vaporizer cup

B= Weight of the vaporizer cup + sample before heating

C= Weight of the evaporator cup + preheated sample

5. Determination of Ash Levels

Weigh the empty crucible (B), then add 2-3 grams of extract to the weighed crucible (C) and gently heat until incandescent. Gradually increase the temperature to 600 ± 25°C until the sample is completely carbon-free. Allow it to cool in a desiccator, then weigh the ash (A). The ash content is calculated as a percentage of the initial sample weight (Ministry of Health, 2000).

% ash content =((A-B))/C x 100%

Information:

A = Cruising weight + extract after training (grams)

B = Weight of empty cruising (grams)

C = Initial sample weight (grams)

6. pH Examination of Extract

By using a pH meter. The instrument is pre-calibrated with a pH 4 solution and a pH 7 solution. Then the electrodes are washed with aquadest and dried with a tissue. The pH measurement of the condensed extract was carried out by diluting 1 gram of condensed extract with aquadest with 10 ml In an appropriate container, the electrode is immersed,

allowing the reading to stabilize. The value displayed on the pH meter represents the pH level of the extract.

7. Phytochemical Screening Test

Thick extract of sungkai leaves (*Peronema canescens* Jack), A sample weighing 0.5 grams was measured, followed by the addition of 5 mL of distilled water and 5 mL of chloroform. The mixture was then vigorously shaken and allowed to settle until two separate layers were formed: an aqueous layer and a chloroform layer. The aqueous layer was utilized for testing flavonoids, phenolics, and saponins, whereas the chloroform layer was designated for analyzing terpenoids, steroids, and alkaloids.

a. Flavonoid Test (Cyanidine Test Method)

Apply a layer of water 1-2 drops, drip on the drip plate and then add Mg and HCl powder (p), the formation of a red color indicates the presence of flavonoids.

b. Phenolic Test

Take a layer of water 1-2 drops, drip on the drip plate and then add $FeCl_3$ reagent, the formation of a blue color indicates the presence of phenolic content.

c. Saponin test

Shake a layer of water vigorously in a test tube. The presence of a lasting foam for about 15 minutes suggests the existence of saponins.

d. Terpenoid and Steroid Tests

Take a small layer of chloroform filtered with norite, take 2-3 drops of filtrate and let it dry on the drip plate, after drying add anhydrous acetic acid and concentrated sulfuric acid (Lieberman-Bouchard Reagent), the formation of blue or green color indicates the presence of steroids, while when red color is formed indicates the presence of terpenoids.

e. Alkaloid test (Culvenore-Fitsgerald Method)

Use a small layer of chloroform, then add 10 ml of 0.05 N ammonia chloroform, stir gently, add a few drops of$_{H2SO42N}$, then beat gently, let separate. An acid layer is added to A positive alkaloid reaction with a few drops of Mayer's reagent is indicated by the appearance of a white haze or precipitate clumps.

Anti-Inflammatory Activity Test with Red Blood Cell Membrane Stabilization Method

a. Preparation of Extract Concentration (*Peronema canescens* Jack) and Ibuprofen

The parent solution of the extract and ibuprofen were each made at a concentration of 1000 ppm by weighing 50 mg of extract and 50 mg of ibuprofen then each dissolved in isosaline solution up to 50 ml. Then the parent solution of sungkai leaf extract made into several series of concentrations, namely 15, 30, 60 and 120 ppm. Ibuprofen was used as a comparison compound with serial concentrations of 15, 30, 60 and 120 ppm.

b. Making Red Blood Cell Suspension

The blood used is goat blood, because of its high sensitivity to antigens and is also easier to obtain than the blood of other animals. For the red blood cell membrane stabilization test, 10 ml of blood was taken by accommodating the blood of slaughtered goats and then directly inserted into a vacutiner tube containing EDTA, then inserted into a centrifugal tube. It is then centrifuged at 3000 rpm for 10 minutes at 27^{O}C. The supernatants formed are separated using a droppipette. The remaining blood cell deposits are then washed with isosaline solution and centrifuged again. The process is repeated approximately 4 times or until isosalin is clear. The volume of blood cells was measured and resuspended with isosaline so that a suspension of red blood cells with a concentration of 10% v/v was obtained by mixing 2 ml of red blood cells with 18 ml of isosaline solution. The suspension of blood cells is stored at a temperature of 4°C if not used (Oyedapo *et al.*, 2010).

Activity Test of Sungkai Leaf Extract (*Peronema canescens* Jack) on Red Blood Cell Membrane Stabilization.

The solutions used in the activity test of Sungkai leaf extract (*Peronema canescens* Jack) **in**the stabilization of red blood cell membranes are as follows:

a. Preparation of Test Solution

The test solution consisted of 1 ml of phosphate with pH 7.4 (0.15 M), 0.5 mL of red blood cell suspension, 1 mL of sample solution and 2 mL of hyposaline.

b. Preparation of Comparative Solution

The comparator solution consisted of 1 mL of 7.4 pH (0.15 M) phosphate, 0.5 ml of red blood cell suspension, 1 mL of Ibuprofen solution and 2 ml of hyposaline.

c. Control Solution

The positive control solution was prepared using 1 mL of DAPAR phosphate buffer (0.15 M, pH 7.4), 0.5 mL of red blood cell suspension, 1 mL of isosaline, and 2 mL of hyposaline.

These solutions were then incubated at 37°C for 30 minutes before being centrifuged at 5000 rpm for 10 minutes. The supernatant was collected, and the hemoglobin concentration was determined using a UV/Vis spectrophotometer at a wavelength of 577.00 nm.

The stability percentage of the red blood cell membrane is then determined using the following formula.:

% Stability = 100 - [(Control Abs. Lar.-Sample Absorbance)/(Control Absorbance)] X 100%

Reslut and Discussion

This research aims to identify the anti-inflammatory effect of ethanol extract of *Peronema canescens* Jack on the stability of erythrocyte membranes. Sungkai leaf extraction is used by the maceration method.

The viscous extract obtained was 179.41 grams with a weight of 1.475 g from the dry sample and a yield of 12.16% from the sample. Distinctive smell of sungkai, pH of the extract 4.77. The solubility of the extract is 96% alcohol soluble. The result obtained from drying shrinkage was 9.05% and the result from ash content was 3.99%. For phytochemical examination, The findings indicated that the ethanol extract of Sungkai leaves contains flavonoids, steroids, and alkaloids. phenolics.

In this study, using the red blood cell membrane stabilization The in vitro method for evaluating anti-inflammatory activity is based on the stabilization of red blood cell membranes. analogous to the lysosomal membrane (Shenoy *et al*., 2010). Lysosomes have activities that can affect the inflammatory process, so the stabilization of the lysosomal membrane is important in limiting the inflammatory response, by preventing the release of enzymes from within the lysosomes during the inflammatory process (Kumar *et al*., 2012). The stability of red blood cells can be seen when red blood cells are induced by a solution that can cause hemolysis. This causes the transfer of fluid from a low concentration to a higher concentration, and an increase in membrane volume, thus triggering membrane damage characterized by hemolysis. The magnitude of hemolysis that occurs in red blood cell membranes induced by hypothetical solution is used as a measure to determine the anti-inflammatory activity of the extract (Kumar, 2011

The test solution used contained hypotonic solution as a hemolysis inductor, a suspension sample of 10% red blood cells and a test compound in the form of sungkai leaf extract at concentrations of 15, 30, 60 and 120 ppm. The control solution was used as an indicator of the occurrence of hemolysis of 100% where the test compound was replaced with isosaline. Each solution was incubated for 30 minutes at 37^{o}C to give the extract absorption time and see the effect of the sample on red blood cells. Then each solution is centrifuged at 5000 rpm for 10 minutes to separate the parts of red blood cells that are still normal and the parts of red blood cells that have been lysed. Normal red blood cells will form deposits, while lyzed red blood cells will be in the supernatant part which is then separated.

The results of the analysis of test samples that have anti-inflammatory activity can be seen from the decrease in hemoglobin absorbance in the test solution mixture. From the measurement results, the average absorbance value of sungkai leaf thick extract with concentrations of 15, 30, 60 and 120 ppm respectively was 0.594, 0.409, 0.378 and 0.338 and the average absorbance value of ibuprofen with concentrations of 15, 30, 60 and 120 ppm was 0.460, 0.387, 0.306 and 0.221 respectively while for the control the average absorbance value was obtained which was 0.607. Absorbance measurements were carried out at a wavelength of 577.00 nm and Ibuprofen was used as a comparison because it is a non-steroidal anti-inflammatory drug.

Based on the results of the measurement of absorbant values obtained, it can be seen that with the increase in the concentration of sungkai leaf extract, the lower the measured absorbant value. Conversely, the greater the concentration of the compound tested, the less the amount of hemoglobin measured indicates the less lysis of red blood cells and thus the greater the ability of the red blood cell membrane to stabilize. After measuring the absorption value, the percentage of stability is calculated.

The percent stability results show that the higher the concentration, the greater the percent of the ability to stabilize.

As presented in table 1 and figure 1.

Table 1. Percent stability of ethanol extract of sungkai leaves, ibuprofen and control solution

Concentration	**% Average stability**
Sungkai 15 ppm	2,24
Sungkai 30 ppm	32,61
Sungkai 60 ppm	37,72
Sungkai 120 ppm	44,31
Ibuprofen 15 ppm	24,1
Ibuprofen 30 ppm	36,2
Ibuprofen 60 ppm	49,4
Ibuprofen 120 ppm	63,4
Kontrol	0,98

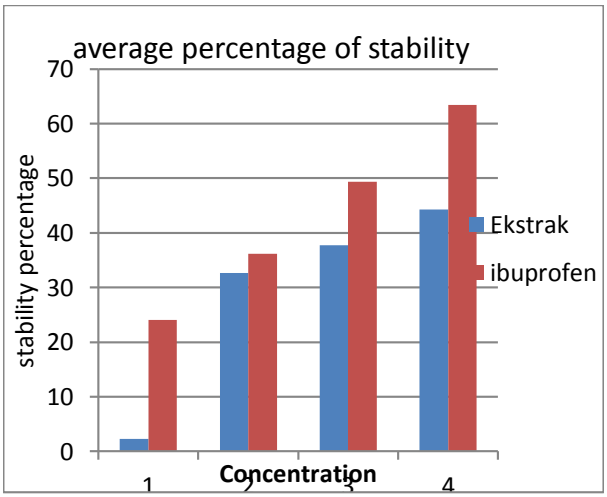

Figure 1. Comparison graph between the variation in the concentration of ethanol extract of sungkai leaf and the variation in the concentration of ibuprofen on the percentage of stabilization of red blood cell membrane

Information:

1. Sungkai and ibuprofen 15 ppm
2. Sungkai and ibuprofen 30 ppm
3. Sungkai and ibuprofen 60 ppm
4. Sungkai and ibuprofen 120 ppm

According to the outcomes of the one-way ANOVA test on the variation in the concentration of ethanol extract of sungkai leaves, ibuprofen and the control solution showed that the ethanol extract of sungkai leaves had an anti-inflammatory activity which was characterized by a significant value of $p<0.05$ which meant that there was a significant difference between the variation in the concentration of ethanol extract of piladang leaves, ibuprofen and also the control solution.

After the ANOVA test, it was followed by the Duncan test. The Duncan test is a follow-up test to test the difference between all variations in the concentration of ethanol extract of sungkai leaves, ibuprofen and the control solution. From the results of the Duncan test, it was concluded that there was a significant difference between each of the concentrations tested

The red blood cell membrane stabilization method can provide an overview of anti-inflammatory activity as seen from the ability of the test material to stabilize the red blood cell membrane so that it provides a good percentage of stability results. The percentage obtained is based on the absorbance value of the measurement results using UV-Visible spectrophotometry. Increased concentration of test material provides better results. The higher the concentration of ethanol extract of sungkai leaves (*Paronema canescens* jack) given, the smaller the absorbance value obtained which indicates that less lysis occurs in the red blood cell membrane, so that less hemoglobin absorbs light from UV-Visible. The decrease in the absorbance value results in an increase in the percentage stability of the red blood cell membrane. The higher the stability percentage, the better the Inflammation-reducing activity of the ethanol extract of sungkai leaves (*Paronema canescens* Jack) and it is also known that the concentration level affects the anti-inflammatory activity produced**.**

Conclusion

Based on the results of the anti-inflammatory activity test of sungkai leaf ethanol extract (*Paronema canescens* Jack) with the red blood cell membrane stabilization method in vitro, the conclusion was obtained that the Inflammation-fighting activity of sungkai leaf ethanol extract (*Paronema canescens* Jack) using the red blood cell membrane stabilization method and vThe concentration of ethanol extract of sungkai leaves (*Paronema canescens* Jack) has an effect on anti-inflammatory activity with an increase in the percentage of stability of red blood cell membranes following an increase in the concentration of ethanol extract of sungkai leaves.

Suggestion

It is recommended that the next researcher to conduct an anti-inflammatory activity test of sungkai ethanol extract (*Paronema canescens* Jack) by using *an invivo* method such as the artificial edema formation method to further ensure the Soothing for inflammation activity of sungkai leaf ethanol extract.

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