Anti-inflammatory Activity Test for Ethanol Extract Moon Flower (*Tithonia diversifolia*) Leaves to Male White Mice

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Abstract: Introduction Hibiscus leaves (*Tithonia diversifolia*) are wild plants. Leaves, root bark and stems of the moon are parts that can be used for traditional medicine. This research was conducted with the aim to determine the effect of ethanol extracts of the moon flower (*Tithonia diversifolia*) as an anti-inflammatory Methods. The method used was artificial edema of white male male foot mice with 1% carrageenan induction of 0.05 ml as an edema maker and as a positive control using Na-Diclofenac. This research was a kind of pure experimental research. Data obtained to find the percentage of inflammation inhibition power. Data distribution was analyzed by the Kolmogorov-Smirnov test, followed by the ANOVA test and the LSD test with a confidence level of 95%. The research results showed that lunar leaves have an anti-inflammatory effect expressed by the% inhibitory inflammation at the lowest dose of 50 mg / kg bw followed by 100 mg / kg bw and 150 mg / kg bw have the highest percentage of inflammation inhibition. In the ANOVA test showed a significant difference in each treatment group at the hours of 1 to 6 hours significantly different at the test level ($\alpha \leq 0.05$). Discussion was expected to be able to identify the potential antioxidant activity of the leaves of the moon flower (*Tithonia diversifolia*) by chromatography.

1 INTRODUCTION

The use of traditional medicine has become a habit practiced by almost all countries in the world. Over the past decade, the use of traditional medicines has grown rapidly. The development of traditional medicine continues to be done as health care for the poor in developing countries (Karamian et al, 2013). Traditional medicine was widely used by the community to maintain health and was in great demand because it is inexpensive and its availability is affordable for the community, especially in villages or small towns where health centers are scarce. Compared to modern medicine, traditional medicine has several advantages, namely its side effects were relatively low. It must be realized that there were dangerous traditional medicinal ingredients if their use exceeds safe dosages and concentrations (Katno and Pramono, 2005). The use of traditional medicines including herbs can be beneficial for health maintenance, prevention and treatment of diseases (Aditama, 2014).

Indonesia is a tropical country with plant potential that has been traditionally used as traditional medicine and has been an Indonesian culture since centuries ago until now. Indonesia with a tropical climate causes fertile soil so that many types of plants can grow. Among the various types, several types of plants have medicinal properties (Hariana, 2013). Moon flower (*Tithonia diversifolia*) is a plant species that belongs to the Asteraceae family. The part that was used from lunar plants as a source of chemicals, which is used for traditional medicine is usually the leaves, but can also use the root bark and stem. The leaves of this month's flower plants contain alkaloids, terpenoids, flavonoids, saponins, tannins, and polyphenols. The benefits of lunar leaves are traditionally usually used as a medicine for stomach aches, bloating, diarrhea and used as a wound and anti-inflammatory drug (anti-inflammatory) (Dalimartha, 2000).

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Inflammation is a process that involves a series of events that can be caused by various stimuli such as infectious substances, ischemia, antigen-antibody interactions, and injuries due to heat or other physical injuries (Goodman and Gilman, 2014). Each type of stimulus has common signs of inflammation such as swelling, pain, redness, heat and loss of cell function that causes discomfort for sufferers so treatment therapy was needed to overcome them, by using modern drugs or medicines derived from plants (Supriyatna et al., 2015).

Therapy that can be given to patients with inflammatory complaints can be given with Non-Steroid Anti-Inflammatory Drugs (NSAIDs) Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) that can relieve symptoms, maintain cell or tissue function and slow down or stop processes that damage tissue (Katzung et al., 2017). Non-steroidal Anti-Inflammatory Drugs (NSAIDs) which are widely circulating are modern drugs that can inhibit various inflammatory mediators (Pinzon, 2007). The use of NSAIDs orally generally cause various side effects problems such as central nervous system (headache), cardiovascular (fluid retention, hypertension), gastrointestinal tract (abdominal pain, dysplasia), hemotology, liver, lung, skin and kidney (Katzung, 2017) . Therefore the use of anti-inflammatory drugs from plants can be used as an alternative treatment with relatively smaller side effects than modern medicine (Kinanti, 2016).

Based on the research of Widia, et al. (2016) showed that the formulation of ethanol extract of moon leaves leaves has the potential to inhibit Staphylococcus aureus using well diffusion method. Hanifa, (2015) showed that the antioxidant activity of ethanol extracts from lunar leaves contained total flavonoid levels of 4.209 mg QE / gram extract. Meanwhile, Suherman (2013) reported that testing the antioxidant activity of *Tithonia diversifolia* leaf extract produced IC 50 against free radical DPPH of 27.88 μg / ml. The results of the separation of lunar leaf extract by thin layer chromatography obtained flavonoid compounds namely 5,7,8,3',4'-pentahidroksiflavonol or 5,6,7,3',4'-pentahidroksiflavonol (Aisha, et al., 2015).

Flavonoid compounds have anti-inflammatory activity by inhibiting the release of serotonin and histamine to the site of inflammation and inhibiting the synthesis of prostaglandin from arachidonic acid by inhibiting the action of cyclooxygenase (COX) (Hasanah, 2011). Based on Ramadhani and Sri's research (2017) flavonoid compounds have anti-inflammatory activity. The strength of the anti-inflammatory effect was indicated by the percentage of edema inhibition. Based on the description above, the moon flower leaves contain flavonoids which were expected to be used as new drugs in anti-inflammatory treatment. The author conducted research to determine the anti-inflammatory effects of ethanol extracts of lunar leaves in male white mice carrageenan-induced.

2 RESEARCH METHODS

This type of research used in this study was purely experimental methods. The research phase includes sample preparation, sampling, preparation of experimental animals, simplicia characteristics, phytochemical screening, extraction methods and testing of anti-inflammatory effects on white male mice. The basis of this method was to make an edema on the sole of the back foot of the mouse using 1% karegenan. The research data were analyzed with Analysis of Variance (ANOVA) with 95% confidence so that it can be known whether the differences obtained are significant or not, if there are significant differences followed by the Least Square Difference test (LSD). The research site was carried out at the Lubuk Pakam Institute of Health Chemistry Laboratory in the manufacture of reagent solutions, characteristics of phytochemicals and phytochemical screening, simplicia extraction processes and anti-inflammatory testing. The time of the study was carried out in March - May 2019.

The tools used in this study are laboratory glassware, UgoBasille®- Plethysmometer, mortar and stamper, injection syringe, mouse cage, digital camera, analytical balance, vacuum rotary rotary evaporator, oral sonde, tissue rolls, labels, blenders, animal scales. The materials used in this study were lunar leaves, chemicals used sodium diclofenac (PT.IndoFarma factory), Carboxy Methyl Cellulose (CMC), λ-carrageenan, sodium chloride solution 0.9%, Pb (II) acetate, iron (III) chloride P, mercury (II) chloride, potassium iodide, iodine, α-naphthol, nitric acid, bismuth nitrate, ether, clofrom, isopropanolol, ethanol, methanol, sodium sulfate anhydrous, ethyl acetate, magnesium powder, bismuth nitrate, ether, chloroform , isopropanolol, ethanol, methanol, sodium sulfate anhydrous, ethyl acetate, magnesium powder, zinc powder, hydrochloric acid P, ether, sulfuric acid P and distilled water.

The experimental animals used were white male mice with a body weight of 20-25 g and 8 weeks of age of 25 animals. Sample was done purposively, without comparing with the same material from

other regions. Samples taken are old and flowering. Single leaf, carved until half the length of the leaf bone, jagged, alternating, pointed tip and base, pinnate, and green. The sample used was the moon flower leaves (*Tithonia diversifolia)* obtained from Tigaras, Kec. Dolok Pardamean, Kab. Simalungun, North Sumatra Province.

Leaves that have been taken were washed using running water drained, then weighed wet weight. Then dried for three days, then sorting was dry and chopped. Furthermore, it was dried in a drying cabinet until it was brittle after it was blended into powder, weighed then put into a tightly closed plastic bottle container and stored at room temperature. To see the characteristics of simplicia, macroscopic and microscopic examination was performed. The phytochemical screening of samples as follows:

Alkaloid: 0.5 gram of simplicia powder was weighed, added 1 ml of 2 N hydrochloric acid and 9 ml of distilled water, heated over a water bath for 2 minutes, cooled and filtered, the filtrate was used for the alkaloids test. 3 test tubes were taken, then 0.5 ml of filtrate were put into each test tube. In tube I: 2 drops of Mayer's reagent are added, forming white or yellow lumpy deposits, in tube II: 2 drops of Dragendrof reagent are added, a brown or orange-brown precipitate is formed, in tube III: added 2 drops of Bouchardat reagent, will form a brown sediment to black. The alkaloid is positive if there is sedimentation or turbidity in two or three of the above experiments (MOH RI, 1995). **Saponin**: 0.5 gram of simplicia powder was weighed, put in a test tube, added 10 ml of hot distilled water, cooled, then shaken vigorously for 10 seconds. Saponin is positive if a stable foam is formed not less than 10 minutes as high as 1 to 10 cm and with the addition of 1 drop of 2 N hydrochloric acid the foam does not disappear (MOH RI, 1995). **Tannin**: As much as 0.5 gram of simplicia powder was weighed, then mixed with 10 ml distilled water, the filtrate was diluted with water until it was colorless. The solution is taken as much as 2 ml and added 1-2 drops of reagent iron (III) chloride 1%. If there is a blue or blackish green color indicates the presence of tannins (Farnsworth, 1966).

Preparation of Ethanol Extract Simplicia powder was extracted by maceration using 70% ethanol solvent. Procedure: Samples were weighed as much as 500 grams, then put into maceration containers. Soaked with solvent until completely submerged and then covered and stored at room temperature. Stirring once a day for five days. After that the solvent is separated from the pulp by pouring the solvent in another container, and the remaining solvent in the pulp is mixed and filtered. To ensure the extraction process takes place perfectly, the pulp that has been kneaded was soaked again using a new ethanol solvent. Left for two days while stirring every day, then kneaded and filtered. Do the same treatment until the solvent is colorless. All maserates are combined and evaporated using a rotary evaporator at ± 400C until a thick extract is obtained.

Preparation of Control Test Materials and Comparative Medicines Hibiscus leaf extract with a dose of 50 mg / kg bw, 100 mg / kg bw, 150 mg / kg bw (test material), Diclofenac sodium suspension dose 6.5 mg / kg bw (positive control), CMC suspension 0.5 % (negative control), carrageenan 1% (induction). The preparations consist of: Making a 0.5% CMC suspension: A total of 0.5 g CMC was sprinkled evenly into a mortar containing 35 ml of hot distilled water, allowed to stand for 15 minutes until obtained a transparent mass, crushed to form a gel and then diluted with a little water, put in a 100 ml flask, then added distilled water to the mark line. Preparation of diclofenac sodium suspension: 6,5 mg of diclofenac sodium was added and then crushed with the addition of 0,5% CMC suspension until homogeneous, put into 10 ml flask, then added 0,5% cmc suspension to the mark line. Preparation of ethanol extract suspension: Weighed 50 mg, 1000 mg, 150 mg extract of moon flower leaves. Each crushed by adding 0,5% CMC suspension until homogeneous, put into a 10 ml flask, sufficient to the mark with 0,5% CMC suspension. Praparation of inflammation indicator: Weighed 100 mg of carrageenan lambda, then homogenized with 0,9% sodium chloride solution, then put in a 10 ml flask, then supplemented with 0,9% sodium chloride solution until the mark line was than allowed to stand and incubated at 37^0C for 24 hours.

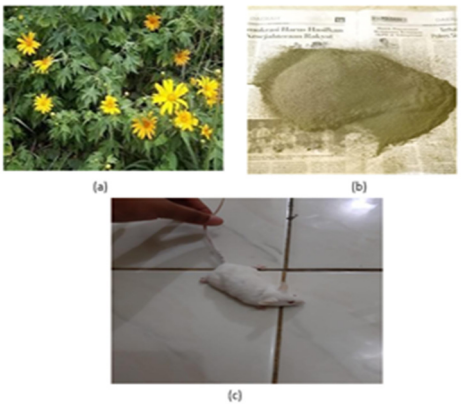

Figure 1: Experiment Sample: (a) *Tithonia diversifolia* (b) Simplisia Powder (*Tithonia diversifolia*), (c) Animal Research.

2.1 The Anti-inflammatory Procedure Were Tested with the following Scheme

Before testing, mice were fasted for 18 hours while still being given a drink. Mice were grouped into 5 groups, namely the negative control group (0.5% CMC suspension), the test material group (three doses of lunar extract extract suspension) and the positive control (diclofenac sodium). On the day of the test, each animal was weighed and marked on its left leg and tail, then the left leg of the mice was put in a cell containing a reservoir solution that had been prepared beforehand until the liquid rose at the upper boundary line, the pedal was then held, recorded figures on the monitor as the initial volume (Vo), which is the foot volume before treatment is given. The preparation is given orally with a volume of giving to mice as much as 1 ml in accordance with the treatment group as follows: Group I: 5 mice were given a 0.5% w / v Na-CMC suspension orally as a negative control; Group II: 5 mice were given orally diclofenac sodium solution (positive control); Group III: 5 mice were given orally extracts of hibiscus leaves at a dose of 50 mg / KgBB; Group IV: 5 mice were given orally extracts of hibiscus leaves at a dose of 100 mg / kg; dan Group V: 5 mice were given lime leaves extract at a dose of 150 mg / KgBW orally. One hour later, each mouse was induced with 0.05 ml of carrageenan 1% intraplantar then measured the initial volume of the mice's feet. After that measured the volume of mice edema feet after treatment every interval of 1 hour for 6 hours. The edema volume is determined based on the increase in mercury in the plathysmometer.

3 RESULTS AND DISCUSSION

Macroscopic examination results of fresh moon flowers are leaves single, etched to half the length of the leaf bone, jagged, alternating, leaf length 26-32 cm, width 15-25 cm, tip and base of pointed leaves, pinnate, green leaves. Microscopic examination results showed fresh moon flowers the presence of multicellular single hair closures, cuticles, upper epidermis, palisades, spiral trachea, spongy tissue, lower epidermis, and leaf mouth Diitic type. This Phytochemical screening of the simplicia leaves of the moon flower to show the class of secondary metabolite compounds contained therein. The examination carried out on the simplified powder of the moon flower is the examination of the group of alkaloid compounds, flavonoids, saponins, and tannins. The results of phytochemical screening of the simplicia leaves of the moon flower are alkaloids (-), flavonoids (+), saponins (-), and tannins (+). Description (+) positive means it contains a class of compounds and (-) negative means that it does not contain compounds. Extraction result of moon flower leaves are 500 gram sample weight, 64 gram extract weight, solvent volume (ethanol 70 %) 3 liters, and soaking time 5 x 24 hours.

Inflammation is a disorder that is often experienced by humans and animals that cause pain in the surrounding area. So the need for prevention or treatment to reduce pain, fight or control pain due to swelling. In this anti-inflammatory study the method used was the formation of artificial edema on the soles of mice's feet using carrageenan as an induction of edema. This method was chosen because it is one of the methods of testing anbtiinflamasi activity that is simple, easy to do and often used. The use of carrageenan as an inducer of edema has several advantages including not leaving a scar, not causing tissue damage and giving a more sensitive response to anti-inflammatory drugs (Fitriyani, 2008).

Carrageenan as an irritant compound induces cell injury through the release of mediators that initiate the inflammatory process. In ssat release of inflammatory mediators occurs maximal edema and lasts several hours. Inflammation induced by carrageenan is characterized by increased pain, swelling, and prostaglandin synthesis up to 4-5 times. Udem caused by carrageenan induction lasts for 6 hours and gradually decreases within 24 hours (Taufiq, 2008). EEDKB anti-inflammatory activity testing used 25 test animals, with 5 treatment groups. The group consisted of positive control given Na diclofenac at a dose of 6.5 mg / kgBW orally, negative control given CMC Na treatment 0.5% orally, the extract treatment group dose 50 mg / kgBW, the extract treatment group dose 100 mg / kg kgBW, and the 150 mg / KgBW dose extract group. The mice were fasted for ± 18 hours, then the mice were weighed marked on the tail and left ankle of the rat. Before each group was given ethanol extract of lunar leaves, the volume of mice's feet was measured first as the initial volume (Vo). After that, each group was given ethanol extracts of lunar leaves ie group I was given a 0.5% Na-CMC suspension, group II was given a diclofenac sodium suspension of 6.5 mg / KgBW, groups III and IV and V were each given an EEDKB suspension dose 50, 100, 150 mg / kgBB orally. One hour later, each foot of the mice's foot was injected intraplantar with

0.05 mL of 1% λ-carrageenan solution. Measurements were made using a pletismometer with measurement principles based on Archhimedes' law. After 30 minutes, the measurement is carried out by dipping the feet of the mice into the pletismometer cells that contain special fluid until the solution reaches the upper limit, and the pedal is held. Numbers are recorded on the monitor. The change in liquid volume that occurs is recorded as the volume of the feet of mice (Vt). Measurements were made every 30 minutes for 360 minutes. Changes in mice foot volume, can be calculated percent inflammation in mice feet. Next, a graph of changes in the average inflammation of the feet of mice was made. The percent inflammation group in the feet of mice smaller than the control group showed that the test material was able to suppress inflammation caused by carrageenan. The results of the percent inflammation measurement can be seen in Figure 2:

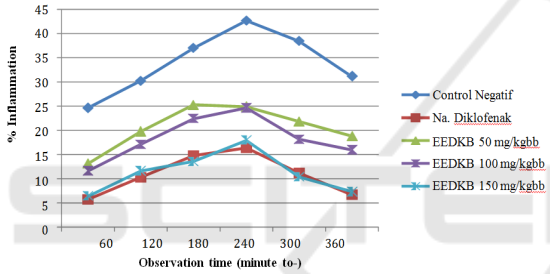

Figure 2: Percent graph inflammation of the average foot mice

In Figure 2 it can be seen that the sodium diclofenac suspension of 6.5 mg / kgBW has a smaller inflammation percentage than EEDKB doses of 50, 100, and 150 mg / kgBW, and the EEDKB dose of 150 mg / kgBW has a smaller percent inflammation than EEDKB doses of 100 and 50 mg / kg body weight. The formation of inflammation by carrageenan produces acute inflammation, and does not cause tissue damage, although inflammation can last for 360 minutes and gradually diminish for one day.

Carrageenan as the cause of inflammation can be influenced by anti-inflammatory drugs. Its response to anti-inflammatory drugs is more sensitive than other irritants (Juheini, et al., 1990). The percentage of mice foot inflammation smaller than the control showed that the diclofenac sodium suspension and EEDKB suspension were able to inhibit inflammation in the mouse feet caused by carrageenan.

The ability to inhibit this inflammation, called inflammation inhibition, can be seen in Figure 3.

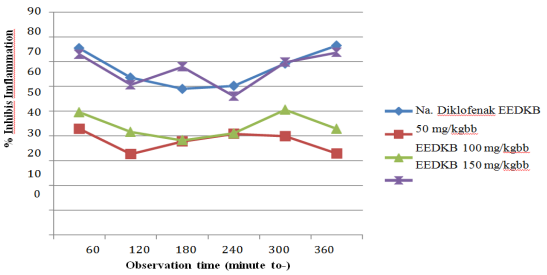

Figure 3: Percent graph of the average inflammation of the feet of mice

In Figure 3 it can be seen that EEDKB 50 mg / kgBW has a smaller percentage of inflammation inhibition than EEDKB 100, 150 mg / kgBW and with diclofenac sodium suspension at a dose of 6.5 mg / kgBW, EEDKB 100 mg / kgBW has percent inflammation inhibition smaller than EEDS 150 mg / kgBW and with diclofenac sodium suspension 6.5 mg / kgBW, and EEDKB 150 mg / kgBW have a smaller percentage of inflammation inhibition than diclofenac sodium suspension at a dose of 6.5 mg / kgBW.

Data obtained in the normality test by the Kalmigorov-Smirnov method to see the distribution of percent data of mice foot inflammation inhibition to the treatment group showed that all treatment groups were normally distributed and not significantly different. Then homogeneity test was performed using the Levene method to see data on the percentage of inflammation inhibition of homogeneous mouse mice or not, the results showed that all treatment groups were homogeneously distributed ($\alpha \geq 0.05$). Because the data meet the homogeneity requirements, ANOVA was continued to look at the average percentage of mice foot inflammation inhibition in the treatment group to see significantly different or insignificant values with a 95% confidence level. Least Square Difference (LSD) test was performed. The results showed that the percentage of mice foot inflammation inhibition throughout the initial volume group in each treatment did not differ significantly, in the induction volume group EEDKB 50 mg / kgBW and 100 mg / kgBW were significantly different, at hour to 1-6 for each treatment is significantly different at the 0.05 test level.

Based on these test results, it can be concluded that the administration of ethanol extracts of lunar leaves at a dose of 50 mg / kg bw, 100 mg / kg bw, 150 mg / kg bw can reduce inflammation in the soles

of male white mice induced by 1% carrageenan. This research has also been carried out by Verawati, et al (2011), it has been reported that the ethanol extract of the moon flower leaves has anti-inflammatory activity. This is seen from the decrease in exudate volume in the back inflammation of female white mice that are given topically. In the test studies the anti-inflammatory effects of ethanol extracts of lunar leaves showed that the effect was dose dependent on increasing certain doses. Anti inflammatory effects can be seen from the content of lunar leaves in which flavonoid compounds have anti-inflammatory activity by inhibiting the release of serotonin and histamine to the site of inflammation and inhibiting the synthesis of prostaglandin from arachidonic acid by inhibiting the action of cyclooxygenase (COX) (Hasanah, 2011).

4 CONCLUSIONS

Ethanol extract of the moon flower (*Thitonia diversifolia*) can provide anti-inflammatory effects. The obtained extracted ethanol of the moon flower leaves (*Thitonia diversifolia*) are 50, 100, and 150 mg / KgBW. The dose of 150mg/kgBW has an anti-inflammatory effect in inhibiting mouse foot edema and the dose of 150 mg/kgBW has smallest percentage of inflammation among the doses used.

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