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# Ethnomedicine Study of Muscle Flour Herbal (Borreria Laevis) In The Tengger Tribe of Bromo East Java as Anti-Inflamation

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**ABSTRACT**

**Objective:** Local wisdom, especially in the ethnomedicine of the Tengger Bromo tribe, East Java, makes a potential that must be developed sustainably. Muscle Flour (Borreria laevis) is a plant that has anti-inflammatory potential in the Tengger tribe. This plant has high ICF and UV tilapia. **Methods:** This study aims to determine the types of plants that have been used by the Tengger tribe have anti-inflammatory activity with the in vitro method of cell membrane stability. Samples were taken from the Tengger Bromo Probolinggo Tribe, extracted by remaceration using 95% ethanol. Characterization includes non-specific parameters and specific parameters. **Results:** Identification of chemical contents includes flavonoids, glycosides, saponins, alkaloids. Testing of anti-inflammatory activity using ethanol extract from muscle flour made concentrations of 250 ppm, 500 ppm, 1000 ppm, and 2000 ppm with the in vitro anti-inflammatory test with the human red blood cell (HRBC) method or cell membrane stability. Subsequently tested in vivo with the carrageenan induction method 0.1 mg kg / BW rats, positive control methylprednisolone 0.072 mg / kg BW rats, at a dose of selected plant extracts 105 mg kg / BW rats, 210 mg kg / BW rats and 560 mg kg / BB mouse.

***Keywords:***
Ethnomedicin, ethanol extract of muscle flour, anti-inflammatory, Tengger tribe, traditional medicine



## 1. INTRODUCTION

Indonesia is a country with the second largest biodiversity in the world after Brazil. More than 20,000 types of medicinal plants. However, only 1,000 types of plants have been recorded and only about 300 types have been used for traditional medicine [1]. Throughout the archipelago, various indigenous tribes living in the vicinity of the forest have used various plant species to maintain health and treat various diseases [2]. Each tribe has local and traditional knowledge in utilizing medicinal plants, starting from plant species, parts used, methods of treatment, to diseases that can be cured and this local knowledge is specific to each tribe, according to the environmental conditions in which each tribe lives[3] However, the process of inheriting local knowledge of traditional medicine is mostly carried out orally and it is feared that the introduction of modern culture to traditional society will cause local knowledge to erode and disappear [4]. This encourages efforts to preserve local knowledge of traditional medicine as early as possible.

Ethnopharmacy is an interdisciplinary science that studies medicinal ingredients, how to use these medicinal ingredients as cultural characteristics in a community group. This study includes the study of: identification, classification and categorization of knowledge of natural substances used as medicine (ethnobiology), preparation of drug preparations (ethnopharmaceuticals), effects claimed to originate from these drug preparations (ethnopharmacology) and social aspects of treatment that affect the use of drug preparations. (ethnomedicin). One of the many ethnic groups in Indonesia whose inhabitants still adhere to the teachings of their ancestors is the Tengger Tribe. The Tengger Tribe area in Probolinggo Regency is a minority area

occupied by some of the Tengger Tribe community. These areas are located in the villages of Ngadas, Jetak, Wonotoro, Ngadirejo, and Ngadisari, Sukapura District, Probolinggo Regency, East Java.

The high price of synthetic drugs and the presence of adverse health side effects have prompted people to reuse traditional medicines [5]. Traditional medicine is also easy to obtain because it grows around the environment in the Tengger tribe. The use of traditional medicines has been passed down from generation to generation and until now, many medicinal plants have scientifically proven efficacy [6]. According to Siswandono and Soekardjo (2000), traditional medicine can be used as the basis for developing new drugs. New drugs that are currently being developed are anti-inflammatory drugs. In an ethnopharmaceutical study of medicinal use in the Tengger tribe, Lumajang and Malang districts, types of diseases that have a high ICF price are associated with types of plants that have high UVs prices to determine which plants have the potential for further research (Ningsih, 2016). According to Batoro and Siswanto (2017) there are 118 types of medicinal plants that are potential to be used to cure 60 symptoms of disease in the Tengger community, including those that have the potential to be anti-inflammatory, such as aseman (Achirantes bidentata Bl.), Kuningan (Widelia Montana Bl), cubung level (Brugmansia candida Pers.) , while dringu (Acorus calamus), poo, dadap leaves, fennel (Foeniculum vulgare), garlic (Allium sativum) for colds and flatulence, guava wer (Prunus persica) for diarrhea. Inflammation or inflammation is a change that occurs in living tissue when it is injured which causes damage to the structure and vitality of the tissue [7]. Inflammation or inflammation is a series of complex changes in tissue resulting from injury and is a mechanism to protect the body.

In the inflammatory reaction there will be release of histamine, bradykinin and prostaglandin. This response occurs in several serious disease conditions such as inflammatory disorders [8]. Red blood cell membrane stabilization has been used as a method of investigating anti-inflammatory activity in vitro. The use of this method is because the red blood cell membrane is similar to the lysosome membrane [9]. The in vitro anti-inflammatory potential was determined based on the HRBC (Human Red Blood Cell) method according to [10]. Erythrocyte membrane stability can be seen by how much erythrocyte hemolysis can be inhibited by the extract. Erythrocyte hemolysis causes hemoglobin to come out of erythrocyte cells so that the solution is reddish. This color will be measured by uv-vis spectophotometry. The more hemolyzed erythrocyte cells, the higher the intensity of the color produced so that the greater the absorbance read by the UV-Vis spectrophotometer. The absorbance of the extract was then compared with the absorbance of a negative control to calculate the percent inhibition.

Conducted an in vitro anti-inflammatory activity test with the cell membrane stability method against the Lantanna camara Linn plant. The test with a positive control of 100 ppm aspirin showed a percentage of inhibition of 93.88% and based on this value it can be said that the aspirin solution is able to inhibit hemolysis of almost all erythrocyte membranes.[11] The percentage stability of the membrane was shown by the extract and the various fractions depending on the concentration and compared with standard drugs (Ibuprofen and Indomethacin). The result is that the ethanol and ethyl acetate fractions can protect erythrocyte membranes effectively. In addition, ethyl acetate fraction provided the highest protection against induced lysis and showed monophasic and biphasic responses at all concentrations tested[12]. [2], in his research, in vivo anti-inflammatory activity test using the 1% carrageenan induction method against muscle flour plants (Stellaria media L.) in the Tengger tribe showed that the dose of 400 mg / kg BW was not significantly different from the positive control for diclofenac sodium dose of 6.5 mg / kg BW. Therefore it is necessary to conduct ethnopharmacological research on plants and traditional medicinal ingredients that have the potential to anti-inflammatory in the Tengger tribe so that the preservation of knowledge and use of traditional medicines is maintained and can be used as a basic reference for developing new medicinal ingredients in anti-inflammatory treatment.

## 2. METHOD

*Study design and research sample*

**Extraction**

Extraction is a separation process based on the mass transfer of chemical components present in a sample of natural materials into the solvent. The principle of this method is based on the distribution of the solute into the solvent. The result of this extraction is called an extract. Some of the extraction methods commonly used for natural organic compounds are maceration, percolation, sox and others. In this study, the extraction was carried out by maceration method with 96% ethanol as solvent. Maceration is the process of extracting plant tissue

using solvents by immersing it several times at room temperature. Researchers used samples taken from plant extracts that had the best Informant Consensus Factor and Use Value values. The dry sample used as much as 3 Kg was put into a maceration bath then immersed in 3000 mL (1:10) 96% ethanol filter liquid, stored for 3 days at room temperature, stirring occasionally, then filtered. The waste obtained is then extracted again. The ethanol extract obtained was then combined and filtered using Whatmann No.1 paper and the obtained filtrate was then concentrated using an evaporator at a temperature below 45 ° C until a thick extract was obtained [13]

**Phytochemical**

Screening The samples used were from plant extracts that had the best Informant Concus Factor and Use Value values with a value close to 1 (one) for the anti-inflammatory activity test. Each type of plant is known and used as a medicinal substance, recorded the local name, the part used, how to use it, and its use. The plants used were determined based on the results of the ethnomedicine study in MMB Batu Malang and comparative studies with medicinal plant research conducted [14] Second, extract samples from each plant that had the best ICF and UV values. The 300-gram dry sample was put into a maceration vessel and immersed in 3000 mL (1:10) 96% ethanol filter liquid, stored at room temperature for 3 days while stirring occasionally, and then filtered. The waste obtained is then extracted again. The ethanol extract obtained was then combined and filtered using Whatmann No.1 paper and the obtained filtrate was then concentrated using an evaporator at a temperature below 45 ° C until a thick extract was obtained [8]

Third, determining the water content of medicinal plant extracts. It was done by weighing the extract by weighing 10 grams and then measuring the water content using the Sterling-Bidwell tool. The time used to measure the water content until there are no droplets on the Sterling-Bidwell tool and wait for 60 minutes to read the scale, then calculate the water content in percent.

Fourth, determination of drying losses. Performed by the gravimetric method. The tool used is Moisture Balance with 3 replications. You do this by entering 2 g of plant extract in a container that has been tared. The container is then put into the Moisture Balance tool. The operation of the tool has been completed if the tool sounds marked with a certain sound, then record the drying loss results.

Fifth, extraction. 1000 g of dry Simplicia were immersed in 10 L of 96% ethanol for 5 days in a maceration vessel. The plant extract was filtered by Whatman No. filter paper. 1 into the vial. The filtrate obtained is then concentrated using an evaporator with a temperature below 45 ° C until a thick extract is obtained [12].

Sixth, test the chemical content of the extract using thin-layer chromatography (TLC). Performed on all plants that have anti-inflammatory activity resulting from ethnomedicine in the Tengger tribe. Flavonoids test with TLC used a stationary phase of silica gel GF254 and the mobile phase used formic acid: ethyl acetate: water: glacial acetic acid (11: 100: 57: 11). The presence of flavonoids is indicated by yellow spots under UV rays of 254 and 366 nm after being sprayed using a routine.

The phenolic test with TLC was carried out by spotting the sample on the GF254 Silica Gel plate. The plate was then put into a chamber containing the mobile phase of n-butanol: acetic acid: water (4: 1: 5) then was removed to the limit, the plate was dried and observed under light UV 254 nm and UV 366 nm with FeCl3 spray reagent. Saponin Test. To determine the presence of saponin compounds, identification was carried out using thin-layer chromatography, the stationary phase of GF 254 silica gel and the mobile phase was chloroform: methanol: water (6: 3: 1). Detected under UV light 254 in yellow and under UV light 366 in green. Anisaldehyde spray reagent with a purple color and blue light spots under ordinary light.

**Anti-inflammatory activity testing**

**Human Red Blood Cell (HRBC) Method**

Preparation of a sterile alsever solution by adding 2 g dextrose, 0.8 g sodium citrate, 0.05 g citric acid and 0.42 g NaCl were dissolved in distilled water to 100 ml at room temperature. Then sterilized using an autoclave at a temperature of 115oC for 30 minutes (Kumar et al., 2012). Preparation of phosphate pH 7.4 (0.15 M) by adding 2.671 g of disodium hydrogen phosphate (Na2HPO4.2H2O) dissolved in distilled water to 100 ml (0.15 M). 2,070 g of sodium dihitrogen phosphate (NaH2PO4. H2O) were dissolved in aquedes until 100 ml (0.15 M) were mixed with 19 ml of NaH2PO4 .H2O (0.15 M) solution at room temperature, then sterilized by autoclaving at 115oC (Oyedapo et al. al., 2010). Preparation of isosalin by mixing 0.85 grams of dissolved NaCl and phosphate pH 7.4 (0.15 M) to a volume of 100 ml at room temperature, then sterilized using autoclave at a temperature of 115o C [15].

The hyposalin preparation of 0.25 grams of NaCl was dissolved in a phosphate kitchen pH 7.4 (0.15 M) to a volume of 100 ml at room temperature, then sterilized by autoclaving at 115oC (Oyedapo et al., 2010). Preparation of red blood cell suspension by taking 10 ml of blood was inserted into a centrifuge tube containing 10 ml of sterile alsever solution, then centrifuged at 3000 rpm for 10 minutes at 27oC, then the supernatant formed was separated using a sterile pipette. The remaining blood sediment is washed with isosalin solution and again centrifuged. The process was repeated 4 times until the isosalin was clear. The volume of blood cells was measured and added with isosalin so that a red blood cell suspension with a concentration of 10% v / v was obtained by mixing 1 ml of blood plus 9 ml of isosalin solution [16].

Testing of extract activity on erythrocyte membrane stabilization. Done by the stages of making a test solution of 1 ml of phosphate buffer pH 7.4 (0.15 M), 2 ml of hyposalin, 0.5 ml of red blood cell suspension and 1 ml of sample solution. Preparation of a positive control solution 1 ml of phosphate buffer pH 7.4 ml (0.15M), 2 ml of hyposalin, 0.5 ml of red blood cell suspension and 1 ml of diclofenac sodium solution. Preparation of a control solution of 1 ml buffer test solution containing phosphate pH 7.4 (0.15 M) 2 ml of hyposalin, 0.5 ml of isosalin solution and 1 ml of sample solution. 1 ml of phosphate buffer pH 7.4 (0.15 M), 2 ml of hyposalin 0.5 ml of red blood cell suspension and 1 ml of isosalin solution. Each solution was incubated at 37oC for 30 minutes and centrifuged at 3000 rpm for 20 minutes. Measurement of the stability of red blood cell membranes using a UV spectrophotometer at a wavelength of 560 nm (Oyedapo et al., 2010). Calculation of the stability of red blood cells using the following formula:

$$\% \text{ stability} = 100 - \left[\frac{\text{Abs Test solution} - \text{Abs Control solution Test solution}}{\text{Abs Negative control solution}}\right] \times 100\%$$

The membrane stability activity test is expressed by the IC50 (Inhibitor Consentration) parameter. The IC50 value is determined from the linear regression equation between the sample concentration and the percentage of membrane stability. Linear regression equation, r table with a confidence level of 0.95. The IC50 price is inversely proportional to the membrane stability activity, namely the greater the IC50 price, the smaller the membrane stability activity, meaning that the concentration required for membrane stability is 50% greater. Preparation of the extract and sodium diclofenac concentration as much as 50 mg of extract and the fraction dissolved in isosalin to 50 ml (1000 ppm) at room temperature. Diclofenac Na was dissolved in 50 ml of isosalin (1000 ppm) at room temperature then the two solutions were diluted to concentrations (50,100,200,400 and 800 ppm).

The effect of giving selected plant ethanol extracts on anti-inflammatory methyl prednisolone was done by calculating the volume of edema. Edema volume is the difference in the legs of rats before and after stirring with 1% injection of suplantar carrageenan. After obtaining the data on the volume of edema, a curve for the comparison of volume versus time was made. Then the AUC (Area Under the Curve) is calculated, which is the average area under the curve which is the relationship between the average volume of edema for each unit of time. With the formula:

$$AUC\ {}_{tn-1}^{tn} = \frac{Vtn - 1 + Vtn}{2}(tn - tn - 1)$$

Information :
Vtn-1 : Average volume of edema at tn-1
Vtn : Volume of average edema in tn

Statistical analysis was used in data processing, previously a hypothesis was tested to determine whether there was a difference in the percentage of edema inhibition in rats' feet from the treatment group and to determine which treatment group had the most optimal anti-inflammatory power, AUC quantitative data between treatment groups was tested for normality. This needs to be done to determine whether the hypothesis testing is carried out by parametric or non-parametric methods. The test criterion is if the significance value is> 0.05, the data is normally distributed and the hypothesis testing is done by using parametric methods, one of which is one-way ANOVA.

## 3. RESULTS AND DISCUSSION

Organoleptic examination of muscle powder plant powder is shown in Table 1.

Table 1. Organoleptic examination results of muscle meal powder

| Material / plant | Checking type | Result |
|---|---|---|
| Muscle flour | Shape | Powder |
| | Color | Brownish green |
| | Smell | Typical |
| | Taste | Negative /tasteless |

Organoleptic examination of plants carried out at the Phytochemical Laboratory of the Setia Budi University, Surakarta. Organoleptic results of muscle flour leaf powder can be seen in table 1 which shows that the herb muscle flour has a brownish green color, a distinctive odor and a tasteless.

Table 2. The results of the characterization of plant extracts based on ICF and UV values

| Plant | Plant part used | Dry powder weighing (grams) | Extract yield (%) | Water content | Shrink extract drying |
|---|---|---|---|---|---|
| Muscle flour | Herbs | 5000 | 6,79 | 8,67 ± 0,31 | 8,96 ± 0,08 |

The part of the plant that is used is the herb muscle flour that is not too old, not too young and still fresh and free from pests. The herbs are washed thoroughly using clean water, then drained then ready to be oven-dried at 50$^{o}$C until dry. The characteristics of good Simplicia are that the color is not much different from the color previously dried, namely the green color according to the original color. Determination of drying losses using the Moisture Balance tool. The aim is to find out the results of the muscle flour herbal powder obtained whether it meets the requirements or not according to the predetermined standards.

The table above shows that from the determination of the drying loss of plant extracts weighed as much as 2 grams, then the drying loss is measured. The time required for measurement is ± 4 minutes for each determination, then the drying loss is obtained in percent (%) units. The average percentage of drying losses in plant extracts is 8.96, this indicates that the drying shrinkage of muscle meal plant extract meets the requirements, which is not more than 10% [17].

The table above shows that the plant extract required for water content determination is ± 10 grams, then the water content is measured using the Sterling-Bidwell tool. The average percentage of plant water content above fulfills the requirements. The extract meets the water content requirements of the extract, namely 5-30% (Voigt, 2004). The high percentage of water content causes changes in the work of enzymes and active substances so that they can reduce the quality of Simplicia and are easily grown by microbes[17].

The extraction method used is the maceration method because it is easy, the tool used is simple, for active compounds that are not resistant to heating and are usually used for simplicia search which contains active ingredients that are easily soluble in solvents and do not contain substances that are easily soluble in solvents and do not contain substances. which expands easily in the dye. The evaporation process is carried out with a rotary vaccum evaporator, the advantage of which is that it can prevent the breakdown or damage of active compounds that are unstable to high temperatures. The result of plant powder extraction produces a thick brownish green extract with a distinctive odor.

Table 3. Results of the chemical content of the extract using color reagents

| Identification | Method | Result | Muscle Flour |
|---|---|---|---|
| Tanin | $KMnO_4$, $FeCl_3$ | A solution was formed | + |
| Flavonoid | Powder Mg BAA | A green solution was formed | + |
| Glikosida | Bourchardad, bond molish | Greenish blue | + |
| Saponin | CHCl3 | The foam is gone | + |
| Alkoloid | Dragendorf, mayer,frohde, $H_2SO_4$,$HNO_3$ | Forms insoluble deposits, brownish yellow, black, orange in color | + |

Note: (+) positive: contains a group of compounds; (-) negative: does not contain a compound group.

The extraction results from the maceration method were then examined for chemical content using a color reaction to check for the presence or absence of flavonoid, saponin and terpenoid-steroid compounds. The results of the identification of the content of plant extract compounds can be seen in the table.

Table 4. Results of erythrocyte membrane stabilization against plant extracts

| Concentration ( ppm) | Sample | Absorbance of Test Solution | Absorbance of Test Control Solution | % Membrane stability |
|---|---|---|---|---|
| 100 | Natrium diclofenac | 0,045 | 0,0272 | 20,28 |
| 2000 | Muscle Flour | 0,059 | 0,045 | 79,71 |
| 1000 | Muscle Flour | 0,065 | 0,041 | 65,21 |
| 500 | Muscle Flour | 0,068 | 0,039 | 57,97 |
| 250 | Muscle Flour | 0,070 | 0,036 | 50,72 |

Red blood cell membrane stabilization has been used as a method to determine anti-inflammatory activity in vitro. This is because the red blood cell membrane is similar to the lysosome membrane[18] which can affect the inflammatory process, so 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. Enzymes in lysosomes released during inflammation (due to activation of neutrophils) will produce various disorders that can be associated with acute or chronic inflammation. The stability of the red blood cell membrane against disturbances induced by hypotonic solutions can also be used as a measure to determine the stabilization of the lysosome membrane [18]. The results of the membrane stability extract test can be seen in the table above. From the data obtained the results of the% stability of the red blood cell membrane from muscle flour. The results showed that the muscle meal plant had the potential for anti-inflammatory activity, therefore it was continued with the IC50 calculation. Table 5 shows the results of the% stability of red blood cell membranes, from the results obtained, the IC50 value was determined by means of linear regression.

Table 5.Results of linear regression on the stability of red blood cell membranes

| Sample | Regresi Linier | Result $IC_{50}$ |
|---|---|---|
| Muscle Flour | Y= 0,0151x + 47,983 | 133,57 ppm |

The $IC_{50}$ value for the stability of the red blood cell membrane was 133.57 ppm in muscle flour. $IC_{50}$ is the concentration which can inhibit 50% of inflammation formation. The $IC_{50}$ value is often used to test for inhibition of inflammation formation. The smaller the $IC_{50}$ value, the more effective the sample is in inhibiting inflammation formation. The anti-inflammatory activity of plant extracts can be seen from a decrease in absorbance in the test solution mixture. The smaller the absorbance value produced, the smaller the hemolysis that occurs, so the greater the anti-inflammatory activity of the sample. The absorbance measurement was carried out at a wavelength of 560 nm. Diclofenac sodium was used as a positive control because it is a non-steroidal anti-inflammatory drug that works by preventing the release of anti-inflammatory mediators so that they can inhibit prostaglandin or cyclooxygenase synthesis. Based on research conducted [19], diclofenac sodium at a concentration of 100 ppm can inhibit red blood cell hemolysis by 51%. Another study conducted [2], also states that diclofenac sodium at a concentration of 100 ppm has the ability to inhibit red blood cell hemolysis by 57.25%. In addition, diclofenac sodium was chosen because it is an anti-inflammatory drug in the NSAID class that is widely used to treat inflammation and is easy to obtain.

The results showed that the concentration of 2000 ppm ethanol extract 96% muscle meal was able to stabilize the red blood cell membrane by 79.71%. At a concentration of 1000 ppm showed the greatest stabilization ability, namely 65.23%. Whereas at a dose of 500 ppm the smallest stability ability was 57.97% and at a dose of 250 ppm the smallest stability ability was 50.72%. This shows that the greater the concentration, the greater the ability of red blood cell stability. This is also proven by statistical analysis, for the initial analysis, a normality test was carried out using the Kolmogorof-Smirnov method to see the distribution of the percent stability of the red blood cell membrane. The results of the analysis showed that all treatment groups were not normally distributed so that the Kruskal-Wallis test was continued. Then proceed with the homogeneity test with

the Levene method to see the percentage of blood cell membrane stability data. The results showed that the treatment group was homogeneously distributed ($p \leq 0.05$) and normally distributed. The different concentrations in the different plant extract treatments proved that increasing the concentration would provide a significant increase in its ability to stabilize the red blood cell membrane which was referred to as the positive control ability (diclofenac sodium) at a concentration of 100 ppm to stabilize the red blood cell membrane. After the measurement, the absorbance data were obtained, then the stability percentage was calculated. The percentage of stability is the ability of a sample to stabilize the red blood cell membrane obtained from the absorption ratio between the absorbance of the test solution and the absorbance of a negative control [20].

The content of chemical compounds in muscle meal of alkaloid (borreline) group, asperulosid, asperulosid acid, hydroxyadoxosid, flavonoid group (kaempferol 3-O-β-D-glucopyranosid, kaempferol 3-O-routineosid, quercetin, routine). Flavonoid compounds will play a role in protecting erythrocyte membranes from hypotonic solutions. The secondary metabolites contained in the extract react in the same amount as the hypotonic solution added to the red blood cell suspension, so as not to damage the erythrocyte cell membrane. While tannin and saponin compounds stabilize the membrane by binding to cations [21] Flavonoid compounds are able to stop the formation and excretion of substances that cause inflammation or inflammation caused by an allergic reaction. The mechanism of flavonoids can go through several pathways, namely directly inhibiting the activity of the COX and lipooxygenase enzymes which cause the inhibition of prostaglandin and leukotriene biosynthesis which are the end products of the COX and lipooksigenase pathways. This can inhibit the accumulation of leukocytes and degranulation of neutrophils, thereby directly reducing the release of arachidonic acid by neutrophils, and inhibiting the release of histamine. In normal conditions leukocytes move freely along the endothelial wall. During inflammation, various endothelial derived mediators and complement factors cause adhesion of leukocytes to the endothelial wall. Administration of flavonoids can reduce the number of leukocytes and reduce complement activation, thereby reducing leukocyte adhesion to the endothelium and resulting in a decrease in the body's inflammatory response [16] [22].

According to Zuhrotun, the mechanism of flavonoids in inhibiting inflammation in two ways, the first inhibits the release of arachidonic acid and lysosomal enzyme secretion from neutrophil and endothelial cells, and the second inhibits the exudation and proliferation phases of the inflammatory process. Flavonoids primarily act on the microvascular endothelium to reduce hypermeability and inflammation. The inhibition of the release of arachidonic acid from inflammatory cells will lead to insufficient arachidonic substrate for the cyclooxygenase and lopooksigenase pathways, which in turn will reduce the number of prostaglandins, prostacyclin, endoperoxides, thromboxane on the one hand and hydroperoxide, hydroxyexosateethaenenoic acid. The anti-inflammatory mechanism of saponins by inhibiting exudate formation and inhibiting vascular permeability inhibits exudate formation and inhibits the increase in vascular permeability [5]. [23] While the presence of phenolic can inhibit inflammation (inflammation) by the mechanism of free radical scavenging and inhibition of the cyclooxygenase enzyme. Phenolic compounds play a role in capturing free radicals that can cause tissue damage and will trigger arachidonic acid biosynthesis to become an inflammatory mediator. The mechanism of alkaloids as anti-inflammatory is by suppressing the release of histamine by mast cells, reducing the secretion of IL-1 by monocytes and PAF on platelets.

Test preparation dosage. Determination of the dose of muscle meal plant extract given to experimental animals was obtained from the previous research doses, namely 75, 150 and 400 mg / g BW of mice after being converted from mice to 200g of mice.

Table 6. Results of dosage determination in test animals

| Claster | Test animal dosage |
|---|---|
| Group I | 105 mg/kg BB |
| Group II | 210 mg/kg BB |
| Group III | 560 mg/kg BB |

Lambda (λ) 1% carrageenan dosage. The dose of carrageenan used in rats was 0.1 ml / kg BW of rats. Methyl prednisolone dosage. Methyl prednisolone was used as a positive control. The dose of methyl prednisolone used in humans was 4 mg converted to rat test animals. The dose for mice was 0.072 mg / kg BW for mice. Testing the anti-inflammatory effect of plant extracts and methyl prednisolone. Testing of the anti-

inflammatory effect of muscle meal extract and methyl prednisolone was carried out on male white rats, the Wistar strain, aged 2-3 months and weighing 200-250 grams. This test was intended to determine the anti-inflammatory effect of plant extracts and methyl prednisolone.

Table 7. AUC results and percent anti-inflammatory power of the control solutions and selected plants

| Test solution - sample | Dose **mg/Kg BB** | **AUC ± SD** | Percent anti-inflammatory power |
|---|---|---|---|
| Negative control (CMC) | - | 2.3260 ± 0.1184 | ~ |
| Positive Control ( metil prednisolon) | 0.072 | 0.3780 ± 0.1188 | 73,04 |
| Muscle Flour | 105 | 0.5500 ± 0.1576 [a] | 76,39 |
| | 210 | 0.4800 ± 0.1538 [a] | 79,27 |
| | 560 | 0.4200 ±0.0900 [a] | 81,55 |

Information [a] is significantly different from negative control ($p < 0.05$)
[b] is significantly different from positive control ($p < 0.05$)

The method used in this study was the induction method of edema on the hind legs of test animals using 1% carrageenan. The reason for choosing the edema induction method is because the measurement is fast, accurate, objective, and easy to do because the observations are visible or easily observed [24]. Carrageenan was chosen as an inflammatory substance because it has special benefits as an irritant compound used in anti-inflammatory drug testing and is an acute inflammation-inducing compound in test animals without causing damage to the inflamed legs of test animals [25]. The mechanism of action of carrageenan as a synergistic inflammation-inducing compound with several inflammatory mediators such as bradykinin, serotonin, histamine, prostaglandins, leukotrienes, and chemotactic agents. Carrageenan induces edema in 2 phases, namely: the initial phase is the release phase of histamine, serotonin, and bradykinin. The final phase is associated with the release of prostaglandins and the presence of cyclooxygenase (COX-2) induction which increases vascular permeability and neutrophil infiltration that produces free radicals that can cause edema, local or systemic inflammation is associated with increased pro-inflammatory cytokines TNF-α, IL-1 , and IL-6 [10]. The percentage of anti-inflammatory power data was statistically analyzed to see that there were significant differences in anti-inflammatory effects between treatment groups. The statistical test performed was the Kolmogorov Smirnov test. The test results obtained were ($p > 0.05$), meaning that the data were normally distributed. Furthermore, the one way ANOVA test was carried out to test the comparative hypothesis on the average of several samples, if the sample consisted of only one category 4]. The result obtained from ANOVA is 0.000 ($p < 0.05$), which means there is a real difference. To find out whether the differences were significant or not between treatment groups, the Post Hock test was performed The percentage of the inflammatory power of muscle flour extract was greater with an increase in the dose of up to 560 mg / kg BW with a positive control comparison of methylprednisolonkan which had a percentage of inflammatory power of 73.04%.

## 4. CONCLUSION

The knowledge or use of traditional medicine in the Tengger Tribe which consists of 5 villages, namely Ngadirejo Village, Ngadas Village, Jetak Village, Wonotoro Village, and Ngadisari Village from 29 sources, 29 types of diseases with 60 traditional recipes and 47 plants, 3 types of animals and 5 natural mineral ingredients. A plant that is used by the Tengger tribe that has the potential to be anti-inflammatory with high UV and ICF values is muscle flour. Flour plant has anti-inflammatory activity with the in vitro method having an IC50 value of muscle flour of 133.57 ppm. Muscle flour plants with the in vivo method have the largest percentage of anti-inflammatory power compared to others, namely at a dose of 105 mg / kg BW of 76.39%, a dose of 210 mg / kg BW of 79.27 and a dose of 210 mg / kg BW of 81, 55%.

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**REFERENCES**


[1] A. Hariana, *Tumbuhan Obat dan Khasiatnya*, 1st ed. Jakarta: Jakarta Penebar Swadaya, 2011.
[2] A. S. Giri, "Tumbuhan Antimikroba Yang Digunakan Masyarakat Suku Tengger," vol. 3, no. 1, p. 9.
[3] A. Fitriyani and L. Winarti, "ANTI-INFLAMMATORY ACTIVITYY OF Piper crocatum Ruiz & Pav. LEAVES," *Maj. Obat Tradis.*, p. 9, 2011.
[4] Departemen Kesehatan Republik Indonesia, *Materia Medika Indonesia Jilid I*. Jakarta, 1977. [Online]. Available: http://ikifa.ac.id/repository-digital/index.php?p=show_detail&id=464
[5] E. M. Kuntorini, "BOTANI EKONOMI SUKU ZINGIBERACEAE SEBAGAI OBAT TRADISIONAL OLEH MASYARAKAT DI KOTAMADYA BANJARBARU," p. 12.
[6] N. Jadid *et al.*, "An ethnobotanical study of medicinal plants used by the Tengger tribe in Ngadisari village, Indonesia," *PloS One*, vol. 15, no. 7, p. e0235886, 2020, doi: 10.1371/journal.pone.0235886.
[7] Y. S. Aziz, R. Setianto, and T. S. Wardani, "Tests for the antibacterial and anti-inflammatory potential of the Asem Tengger plant (Radicula armoracia Robinson) obtained from the ethnomedicine study in the Tengger tribe," *Sci. Technol.*, p. 7, 2021.
[8] V. Kumar, Z. A. Bhat, D. Kumar, P. Bohra, and S. Sheela, "IN-VITRO ANTI-INFLAMMATORY ACTIVITY OF LEAF EXTRACTS OF BASELLA ALBA LINN. VAR. ALBA," p. 4, 2011.
[9] A. F. Shalas *et al.*, "Ethnomedicine Study of Tengger People of Ngadas Village in Malang, East Java, Indonesia: In Search of Antimicrobial Plants," *J. Young Pharm.*, vol. 13, no. 2, pp. 97–106, Jul. 2021, doi: 10.5530/jyp.2021.13.22.
[10] S. C. Chippada, S. S. Volluri, S. R. Bammidi, and M. Vangalapati, "IN VITRO ANTI INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF CENTELLA ASIATICA BY HRBC MEMBRANE STABILISATION," p. 4, 2011.
[11] "Antibacterial Activity and Phytochemical Profile.pdf."
[12] T. Kawarai *et al.*, "Inhibition of Streptococcus mutans biofilm formation using extracts from Assam tea compared to green tea," *Arch. Oral Biol.*, vol. 68, pp. 73–82, Aug. 2016, doi: 10.1016/j.archoralbio.2016.04.002.
[13] Y. Ma, Y. Li, X. Li, and Y. Wu, "Anti-inflammatory effects of 4-methylcyclopentadecanone on edema models in mice," *Int. J. Mol. Sci.*, vol. 14, no. 12, pp. 23980–23992, Dec. 2013, doi: 10.3390/ijms141223980.
[14] G. Mahendran and L.-U. Rahman, "Ethnomedicinal, phytochemical and pharmacological updates on Peppermint (Mentha × piperita L.)-A review," *Phytother. Res. PTR*, vol. 34, no. 9, pp. 2088–2139, Sep. 2020, doi: 10.1002/ptr.6664.
[15] Vijender Pareek, ZA BHAT, and D. Kumar, "International Journal of Phytopharmacology EVALUATION OF ANTI-INFLAMMATORY POTENTIAL OF PETAL EXTRACTS OF CROCUS SATIVUS 'CASHMERIANUS,'" 2012, doi: 10.13140/RG.2.2.18813.67048.
[16] G. Leelaprakash and S. M. Dass, "INVITRO ANTI-INFLAMMATORY ACTIVITY OF METHANOL EXTRACT OF ENICOSTEMMA AXILLARE," p. 8, 2011.
[17] S. Gan Gunawan, *Farmakologi dan Terapi*. 2011. [Online]. Available: http://katalogdpklomboktimur.perpusnas.go.id/detail-opac?id=4788
[18] [*Materia Medika Indonesia Jilid III*. Dirjen Pengawasan Obat Dan Makanan Jakarta, 1979.
[19] E. Kuspraningrum, T. Luth, Y. Yuliati, R. Safa'At, and H. Kuspradini, "Review: The conservation of Tengger indigenous people's traditional knowledge of biological natural resource-based disease treatments," *Biodiversitas J. Biol. Divers.*, vol. 21, no. 11, Oct. 2020, doi: 10.13057/biodiv/d211108.
[20] R. Smd and O. Rostiana, "PENGGALIAN IPTEK ETNOMEDISIN DI GUNUNG GEDE PANGRANGO," no. 1, p. 17, 2007.
[21] Hukmiyah, "Penetapan Kadar Fenol Total, Flavonoid & Alkaloid Serta Uji Aktivitas Antiinflamasi Ekstrak Etanolik Tepung Otot Stellaria Media (L.) Vill)," 2018, [Online]. Available: http://repository.unej.ac.id/handle/123456789/89198
[22] M. A. Barkat, A. Goyal, H. A. Barkat, M. Salauddin, F. H. Pottoo, and E. T. Anwer, "Herbal Medicine: Clinical Perspective and Regulatory Status," *Comb. Chem. High Throughput Screen.*, vol. 24, no. 10, pp. 1573–1582, 2021, doi: 10.2174/1386207323999201110192942.
[23] "The Use of Children's Herbal Mouthwash in Pediatric Dentistry: A Systematic Review," *Int. J. Pharm. Res.*, vol. 12, no. sp3, Feb. 2021, doi: 10.31838/ijpr/2020.SP2.564.
[24] P. Ashok, B. C. Koti, A. H. M. Thippeswamy, V. P. Tikare, P. Dabadi, and A. H. M. Viswanathaswamy, "Evaluation of Antiinflammatory Activity of Centratherum anthelminticum (L) Kuntze Seed," *Indian J. Pharm. Sci.*, vol. 72, no. 6, pp. 697–703, Nov. 2010, doi: 10.4103/0250-474X.84577.
[25] M. Hukmiyah, "FAKULTAS FARMASI UNIVERSITAS JEMBER 2018," p. 95.
[26] Siregar, Rahmah. (2022). Factors Which Influence Incident Hypertension on Pre-Elderly. International Journal of Public Health Excellence (IJPHE). 1. 117-121. 10.55299/ijphe.v1i2.66.