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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

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***Abstract—Purpose*: Local wisdom, especially in the Tengger Bromo tribe, East Java makes a potential that must be developed sustainably. The *Asem Tengger* plant (*Radicula armoracia* Robinson) is a plant that has antibacterial and anti-inflammatory potential in the Tengger tribe. This plant has high ICF and UV values. This study aims to determine the types of plants that have been used by the Tengger Tribe have antibacterial and anti-inflammatory activities with the dilution method and in vitro cell membrane stability. *Methodology*: Samples were taken from the Tengger Bromo Probolinggo Tribe, extracted by maceration using 95% ethanol. Characterization includes non-specific parameters and specific parameters. Identification of chemical contents includes flavonoids, glycosides, saponins, alkaloids. Antimicrobial test by dilution method, each concentration was tested on *Staphylococcus aureus* ATCC 2592, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhi* ATCC 13311, and *Candida albicans* ATCC 10231. The anti-inflammatory activity test using ethanol extract from muscle flour was made up to a concentration 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. *Results:* Based on the antimicrobial test, *Asem Tengger* has the potential to be antibacterial to *Staphylococcus aureus* ATCC 2592 in the water and n-hexane fractions with a value of 2.5% MBC. The anti-inflammatory test showed the results of membrane stability of 57.97% from a concentration of 2000 ppm which shows a number that has the potential to be a reference for potential medicinal plants. *Value*: The *Asem Tengger* plant used by the Tengger tribe has the potential as new antimicrobials and anti-inflammatory**

Keywords—ethnomedicine, anti-inflammatory, antibacterial Tengger tribe

I. INTRODUCTION

In the Tengger tribe with varied natural conditions and biodiversity, the people can make good use of medicinal plants. According to Batoro [3], there are 118 types of medicinal plants that have the potential to be used to cure 60 symptomatic diseases in the Tengger community, including anti-inflammatory such as *aseman* (*Achirantes bidentata* Bl.), Kuningan (*Widelia Montana* Bl), cubung (*Brugmansia candida* Pers.), while *dringu (Acorus calamus*),*dadap* leaves, fennel *(Foeniculum vulgare*), garlic (*Allium sativum*) for colds and flatulence, *guava wer* (*Prunus persica*) for diarrhea.

According to Aziz [1] In the perch community, *Asem Tengger* (*Radicula armoracia* Robinson) in the Brassicaceae family is used as a cough medicine and pain reliever. The leaves are chopped and washed with water, then wrapped in banana leaves and baked in the fireplace until wilted. After wilting, the *Asem Tengger* leaves are eaten directly. Tengger acid is not the same as Tamarind (*Tamarindus indica* L.), *Asem Tengger* is almost the same as Tengger Radish (*Raphanus raphanistrum* L.) which comes from the Brassicaceae family, the difference is in the shape and structure of the flowers. *Asem Tengger* can be used as an antiseptic, stimulant, diuretic, and respiratory tract infection with active ingredients in the herbs *Asem Tengger*, including phenols, essential oils, ascorbic acid, asparagine, peroxidase enzymes, resins, and sugars [2], while the active ingredients in the leaves include kaemferol and quercetin.

Based on the large number of medicinal plants that have been used by the Tengger tribe community and the good potential of *Asem Tengger*, it is necessary to develop new anti-inflammatory anti-microbial drugs that come from natural ingredients. Therefore it is necessary to conduct ethnopharmacological research on plants and traditional medicinal ingredients that have the potential for antibacterial and anti-inflammatory in the Tengger tribe so that the preservation of knowledge and the use of traditional medicines is maintained and can be used as a basic reference for the development of new medicinal ingredients in antibacterial and anti-inflammatory treatment.

II. METHOD

This research is a descriptive study that uses qualitative and quantitative methods. Qualitative methods are used to determine the use of plants, while the quantitative method is used to determine the antibacterial and anti-inflammatory activity of plants known and used by the Tengger Tribe as a drug [1].

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A. Extraction

Sample extraction of each plant that has the best ICF and UV values. A 500-gram dry sample was put into a maceration vessel and immersed in a 96% ethanol 96% liquid (1:10), stored at room temperature for 3 days while occasionally stirring and then filtered. The pulp obtained is then extracted again. The ethanol extract obtained was then combined and filtered using Whatman No.1 paper and the filtrate obtained was then concentrated using an evaporator at a temperature below 45 ° C until a thick extract was obtained.

B. Antimicrobial test

Preparation of plant extract stock using DMSO solvent. Each of these tubes has some dilution concentrations of 40%, 20%, 10%, 5%, and 2.5%. BHI media was added 0.5 ml into each test tube aseptically, the first tube was added 0.5 ml of plant extract for each fraction, then shaken then 0.5 ml of the first tube was taken into the second tube, and from the tube the second 0.5 ml is taken into the third tube and so on until the fifth tube. Bacterial suspension in the BHI medium was put into each test tube as much as 0.5 ml. DMSO as a negative control, antibiotics as a positive control. All tubes were incubated at room temperature for 18-24 hours, then turbidity was observed to determine MIC, which is the lowest limit of clear media tubes or those that gave negative results. Then determine the MBC by inoculating the preparation from the test tube on VJA, BCA, PSA, BSA, and RBC media in a *petri* dish and incubated for 18-24 hours at 37 0C. MBC showed the lowest concentration of VJA, BCA, PSA, BSA, and RBC media which did not show a growing bacterial colony.

C. Anti-inflammatory test

1. Test the stability of the cell membrane

Preparation of the test solution. The test solution (4.5 ml) consisted 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. The positive control solution consisted of 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. The control solution for the test solution consisted of 1 ml buffer containing phosphate pH 7.4 (0.15 M) 2 ml of hyposalin, 0.5 ml of isosalin solution as a substitute for red blood cell suspension and 1 ml of sample solution.Preparation of the negative control solution consisted of 1 ml of phosphate buffer p H 7.4 (0.15 M), 2 ml of hyposalin 0.5 ml of red blood cell suspension and 1 ml of isosalin solution as a substitute for the sample solution. Each of the above solutions was incubated at 37oC for 30 minutes and centrifuged at 3000 rpm for 20 minutes. The supernatant obtained is taken and the hemoglobin content, because hemoglobin is a pigment that forms the color of red blood cells, when red blood cells undergo lysis of red blood cell pigments it can be counted so that the sample cannot maintain its cell membrane. Measurement of the stability of the red blood cell membrane using a UV spectrophotometer at a wavelength of 560 nm [4]. Calculation of the stability of red blood cells with the following formula:

% stability = 100 - × 100%

Then the membrane stability activity test is expressed by the IC50 (Inhibitor Concentration) parameter, namely the concentration of the test compound that inhibits cell membrane lysis by 50%. 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 value 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.

2. Preparation of extract concentrations and diclofenac sodium.

A total of 50 mg of extract and fraction were dissolved in isosalin to 50 ml (1000 ppm) at room temperature. Likewise, diclofenac Na was dissolved in 50 ml of isosalin (1000 ppm) at room temperature. Then the two solutions were diluted into several series of concentrations (50,100,200,400 and 800 ppm).

3. Invivo anti-inflammatory test on male white rats

Treatment of test animals. This study used 25 male white rats (*Rattus norvegicus* L.) which were divided into 5 treatment groups. Each group consisted of 5 mice as replications. The test method used was the modified Winter method [4], artificial edema was induced by injecting 1.0 mL of 1% carrageenan dissolved in a physiological solution (0.9% NaCl) into the rats' feet subplantar. Rakhmawati (1997) reported that at these doses, edema was clearly observed. The test materials given were extracts from selected plants containing saponins, flavonoids, and steroids.

The treatment given to each group is as follows:

CMC 1% negative control (placebo) 0.1 mg / kg BW of rats. Positive control for Methyl prednisolone 0.072 mg / kg BW rats. The ethanol extract of selected plants was 105 mg / kg BW of rats The ethanol extract of selected plants was 210 mg / kg BW of rats. Selected plant ethanol extract 560 mg / kg BW of rats. This study used doses of 105, 210, and 560 mg / kg BW, and it was found that the most effective dose as an anti-inflammatory was 560 mg / kg BW [4]. The initial volume of the rats 'feet was measured before being given the treatment, using a pletismometer, by inserting the rats' feet that were marked at the level of the ankles (up to the mark) on the pletismometer. After all were treated, the measurement was carried out again at 0.5 hours; 1; 2; 3; 4; 5 to 24.

4. Analysis of invivo anti-inflammatory test

The effect of giving the ethanol extract of selected plants 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.

$$Vu = Vt - Vo$$

Information:
Vu: Volume of rat foot edema per time t.
Vt: Volume of rat foot edema after stirring with 1% carrageenan at time t.
Vo: Volume of rat foot edema after stirring with 1% carrageenan.

After obtaining the data on the volume of edema, a curve for the comparison of the volume of edema 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

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each unit of time. With the formula:

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

The percentage of anti-inflammatory power can be calculated with the formula as:

Information:
AUCk: AUC of time-to-time mean edema volume curve for negative control.
AUCp: AUC of mean volume of edema versus time in each treatment group.

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 rat 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 then the data is normally distributed and the hypothesis testing is carried out using the parametric method, one of which is one-way ANOVA. If one-way anova shows a real difference, it can be seen from the test of homogeneity of variances to find out that the variations are the same or not. The criterion for the test of homogeneity of variances is that if the significance is> 0.05, the variance is the same, on the other hand, if the significance value is <0.05, the variance is not the same. If the variants are the same, the next step is the Tukey HSD (Honestly Significant Difference) test with 95% confidence, or with the SNK test or LSD test, while if the variants are not the same, you can continue with the Dunnett's T3 test.

III. RESULT

A. Perch Asem Plant Identification.

The reference used in determining the book [5][6][7][8][9][10], obtained the results of the determination of the *Asem Tengger* plant which aims to ensure that the plants used are correct and appropriate, thus avoiding errors in taking the plants used as samples in the study.

B. Taking and Making Perch Asem Powder.

The plants used in this study were plants obtained from the community yard of the Tengger Tribe, Sukapura District, Probolinggo Regency in April-June 2019. The parts of the plants used in the study were the leaves used for medicine by the Tengger Tribe. Furthermore, the plant part used is separated from the inherent impurities or other unnecessary foreign materials, then the plants are washed with clean water. The purpose of washing plants is to remove soil and other inherent impurities.

The next stage, chopping the leaves of *Asem Tengger* (*Radicula armoracia* Robinson) which is then dried in the oven. Drying is carried out at a temperature of 45ºC to reduce moisture content and stop enzymatic reactions in plants. Drying is done until the leaves are easily crushed by kneading. After drying, it is continued with dry sorting. Dry sorting aims to separate foreign objects, then powder them using a blender to produce dry powder.

B. Maceration of Asem Tengger.

The plant powder used as much as 500 grams was replicated 3 times. 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 searches that contain active ingredients that dissolve easily in solvents and do not contain substances that are easily soluble in solvents and do not contain which easily expands in the irradiation fluid [10]. The solvent used is 96% ethanol. The use of 96% ethanol as a pollutant is because ethanol is non-toxic and easily pulls out the active compound from the cell and can mix with water in various ratios, besides that ethanol has a relatively low boiling property so that it is easily and quickly evaporated [10]. After the filtering results are obtained, the next process is evaporation with a rotary vaccum evaporator, the advantage of using a rotary vaccum evaporator 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 greenish brownish color of the extract. The yield of the extract from *Asem Tengger* (*Radicula armoracia* Robinson) was 12.06 ± 0.22.

C. The results of the antibacterial activity test of the Asem Tengger plant.

Antibacterial activity tests of various leaf extracts of *Asem Tengger* (*Radicula armoracia* Robinson) against Staphylococcus aureus ATCC 2592, Bacillus subtilis ATCC 6633, Pseudomonas aeruginosa ATCC 27853, Salmonella typhi ATCC 13311, and Candida albicans ATCC 10231 were carried out by using the dilution method that was replicated 3 times, lasts for 1 x 24 hours. Ciprofloxacin as a positive control was a broad spectrum bacteriostatic antibiotic that was active against Gram-positive and Gram-negative aerobic and anaerobic organisms. DMSO solvent is used as a negative control, functions as a solvent that quickly permeates the extract epithelium without damaging the cells and is often used in the health sector.

Methanol extract, water fraction, ethyl acetate fraction and n-hexane of *Asem Tengger* leaves were tested for their activity against several test microbes with the liquid dilution method to determine the value of the extract's Minimum Inhibitory Concentration (MIC), and then the test was carried out to determine the Minimum Bactericidal Concentration (MBC) of the extract using the diffusion method to make it solid using the scratch method. The test medium was then incubated at 37ºC for 24 hours in an incubator. The MBC cloudy or transparent colonies on the surface of the test medium. Observation of MBC extract was based on the smallest level that was able to kill fungal growth characterized by the presence or absence of fungal growth from scratches resulting from liquid dilution on solid media.

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Table 1. Animicrobial test results of ethanol extract (*Radicula armoracia* Robinson)

Sample grade (% b/v)	Bacterial growth Ethanol extract									
	Staphylococcus aureus ATCC 25923		*Bacillus subtilis* ATCC 6633		*Pseudomonas aeruginosa* ATCC 27853		*Salmonella typhi* ATCC 13311		*Candida albicans* ATCC 1023	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
40	-	-	-	-	+	√	-	-	+	√
20	-	√	-	√	+	√	+	√	+	√
10	+	√	+	√	++	√	++	√	++	√
5	+	√	+	√	++	√	++	√	+++	√
2,5	++	√	+++	√	+++	√	+++	√	+++	√
K+	-	-	-	-	-	-	-	-	-	-
K-	++	√	++	√	++	√	++	√	++	√

Note: (+) positive: there is bacterial growth, the more + the more cloudy it is; (-) negative: there is no bacterial growth. (√) check: there is bacterial growth

Table 2. Animicrobial test results of water fraction (*Radicula armoracia* Robinson)

Sample grade (% b/v)	Growth of water fraction bacteria									
	Staphylococcus aureus ATCC 25923		*Bacillus subtilis* ATCC 6633		*Pseudomonas aeruginosa* ATCC 27853		*Salmonella typhi* ATCC 13311		*Candida albicans* ATCC 1023	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
40	-	-	-	-	+	√	-	-	-	-
20	-	-	-	-	+	√	-	√	-	-
10	-	-	-	-	++	√	+	√	+	√
5	-	-	-	√	++	√	++	√	+	√
2,5	+	√	+	√	+++	√	+++	√	++	√
K+	-	-	-	-	-	-	-	-	-	-
K-	++	√	++	√	++	√	++	√	++	√

Note: (+) positive: there is bacterial growth, the more + the more cloudy it is; (-) negative: there is no bacterial growth. (√) check: there is bacterial growth

Table 3. Animicrobial test results of ethyl acetate fraction (*Radicula armoracia* Robinson)

Sample grade (% b/v)	Bacterial growth ethyl acetate fraction									
	Staphylococcus aureus ATCC 25923		*Bacillus subtilis* ATCC 6633		*Pseudomonas aeruginosa* ATCC 27853		*Salmonella typhi* ATCC 13311		*Candida albicans* ATCC 1023	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
40	-	-	-	-	+	√	-	-	-	-
20	-	-	-	√	+	√	-	√	+	√
10	-	-	+	√	++	√	++	√	+	√
5	-	-	++	√	++	√	++	√	+	√
2,5	-	√	++	√	+++	√	+++	√	++	√
K+	-	-	-	-	-	-	-	-	-	-
K-	++	√	++	√	++	√	++	√	++	√

Note: (+) positive: there is bacterial growth, the more + the more cloudy it is; (-) negative: there is no bacterial growth. (√) check: there is bacterial growth

Table 4. Animicrobial test results of n-hexane fraction (*Radicula armoracia* Robinson)

Sample grade (% b/v)	Bacterial growth n-hexane fraction									
	Staphylococcus aureus ATCC 25923		*Bacillus subtilis* ATCC 6633		*Pseudomonas aeruginosa* ATCC 27853		*Salmonella typhi* ATCC 13311		*Candida albicans* ATCC 1023	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
40	-	-	-	-	-	-	-	-	-	-
20	-	-	-	√	-	√	+	√	-	-
10	-	-	+	√	+	√	++	√	-	√
5	-	-	+	√	+	√	++	√	+	√
2,5	-	-	+++	√	+++	√	+++	√	+	√
K+	-	-	-	-	-	-	-	-	-	-
K-	++	√	++	√	++	√	++	√	++	√

Note: (+) positive: there is bacterial growth, the more + the more cloudy it is; (-) negative: there is no bacterial growth. (√) check: there is bacterial growth

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1 Ethanol extract,

Shown antibacterial activity Minimum Inhibitory concentration (MIC), which is the smallest level of a sample that is able to inhibit fungal growth characterized by clarity in the tube. The Minimum Inhibitory Level was determined by comparing the clarity between the test solution and the control. In this antimicrobial activity test, the MIC of each test bacterium is different. The sample solution is very thick or cloudy brown in color.

Staphylococcus aureus ATCC 25923 and *Bacillus subtilis* ATCC 6633 were able to inhibit their growth at a concentration of 20%, *Salmonella typhi* ATCC 13311 was inhibited at a concentration of 40% whereas *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicansATCC* 10231 were unable to be inhibited by the ethanol extract of *Asem Tengger* leaves.

Minimum Bactericidal Concentration (MBC)is the smallest level capable of killing the growth of bacteria and fungi characterized by the presence or absence of growth of bacteria and fungi from scratches resulting from liquid dilution on solid media. Based on the results of the antibacterial and anti-fungal activity tests, it was shown that at the final level of 40% *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633 and *Salmonella typhi* ATCC 13311 there was no bacterial growth. marked by the absence of growth on solid media, it was concluded that the MBC of *Asem Tengger* leaf ethanol extract was 40% w / v which was active in the bacteria *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633 and Salmonella typhi ATCC 13311. Observation of the teaching and learning test can be seen in Figure attachment 3, while the results can be seen in 1.

From the results of the MIC and Minimum Bactericidal Concentration (MBC) test, the ethanol extract is more effective in inhibiting and killing bacterial growth in gram-positive bacteria, as shown in Table 1 *Staphylococcus aureus* ATCC 25923, Bacillus subtilis ATCC 6633 has the same value. In gram-negative bacteria, the ethanol extract of *Asem Tengger* is less active. Meanwhile, the fungus *Candida albicans* ATCC 10231 ethanol extract of *Asem Tengger (Radicula armoracia Robinson*) leaves was inactive.

2. Water fraction.

The results of the antimicrobial activity test of the *Asem Tengger* water fraction can be seen in Table 2. In the water fraction where the nature of the solvent is polar, the inhibition and killing power of the *Asem Tengger* leaf water fraction were more active. The MIC value of 5% was shown in the results of the inhibition of the bacteria *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633, MIC 20% in *Salmonella typhi* ATCC 13311 and *Candida albicans* ATCC 1023. Whereas in *Pseudomonas aeruginosa* ATCC 27853 there was no MIC value.

The best MBC value is shown in the results of the *Staphylococcus aureus* ATCC 2592 test. In contrast to the MBC value of the *Asem Tengger* water fraction, *Bacillus subtilis* ATCC 6633 has a MBC value of 10%. The water fraction of *Asem Tengger* showed a MBC value of 20% in the test for the fungus *Candida albicans* ATCC 10231. From the polar solvent used, the more effective the inhibition of antibacterial activity, especially in gram-positive bacteria and fungi.

3 Ethyl acetate fraction.

The test results on the ethyl acetate fraction showed that the MIC value of *Staphylococcus aureus* ATCC 25923 was very good, from 5 test concentrations the results were the same as the positive control, namely there was no bacterial growth shown in the absence of turbidity from each tube. The MIC value of *Bacillus subtilis*ATCC 6633 and *Salmonella typhi* ATCC 13311 was 20%, whereas in *Candida albicansA*TCC 10231 the MIC value was 40%.

The minimum kill level of ethyl acetate fraction of *Asem Tengger* leaves was the most in *Staphylococcus aureus*ATCC 25923 bacteria by 5%. At levels of 40% *Bacillus subtilisATCC* 6633, *Salmonella typhi* ATCC 13311 and *Candida albicans*ATCC 10231 showed the killing power of bacteria and fungi. The semi-polar solvent properties of the ethyl acetate fraction of *Asem Tengger*, give the activity of MIC and MBC *in Staphylococcus aureus*ATCC 25923 bacteria. Test observations can be seen in the attachment of table 3.

4. Fraction hexane.

In non-polar hexane solvents, the activity of inhibition and killing power of perch hexane fraction is better than polar and semi-polar solvents. There is MIC and MBC activity in gram-positive and gram-negative bacteria as well as in the fungus *Candida albicans*ATCC 10231 table 4. *Pseudomonas aeruginosa* ATCC 27853 in ethanol extract, water fraction and ethyl acetate fraction did not show MIC and MBC values. *Pseudomonas aeruginosa* bacteria ATCC 27853 in fraction n hexane, MIC value at 20% and MBC 40%

The Tengger tribe people use *Asem Tengger* as a cough medicine. The leaf (*Radicula armoracia* Robinson) in the Brassicaceae family is used as a cough medicine. The leaves are chopped and washed with water, then wrapped in banana leaves and baked in the fireplace until wilted. After wilting, the *Asem Tengger* leaves are eaten directly. *Asem Tengger* is not the same as Tamarind (*Tamarindus indica* L.), *Asem Tengger* is almost the same as *Tengger Radish* (*Raphanus raphanistrum* L.) which comes from the Brassicaceae family, the difference is in the shape and structure of the flowers. The antimicrobial test of *Asem Tengger* leaves showed differences in the activity of each fraction against bacteria and fungi, the best activity was shown from the n hexane fraction. There is a correlation between knowledge and the use of *Asem Tengger* leaves as medicine, indicated by the value of MIC and MBC in S. aureus. S. aureus has the potential to cause ulcers, acne, pneumonia, meningitis, and arthritits.

D. The results of the anti-inflammatory activity test of the Asem Tengger plant.

1. The test results of plant extract activity on erythrocyte membrane stabilization

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 [11] 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 the lysosomes during the inflammatory process. Enzymes in lysosomes released

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during inflammation (due to activation of neutrophils) will produce various disorders that can be associated with the occurrence of acute or chronic inflammation. The stability of the red blood cell membrane against interference induced by hypotonic solutions can also be used as a measure to determine the stabilization of the lysosome membrane[4]. The results of the membrane stability extract test can be seen in table 5.

Table 5. Erythrocyte membrane stabilization of plant extracts.

Sample (ppm) (Concentration)	Absorbance Test Solution	Absorbance Control Solution	Test (%) Membrane stability
Diclofenac sodium (100)	0,045	0,0272	20,28
Asem Tengger 2000	0,084	0,055	57,97
Asem Tengger 1000	0,088	0,051	44,92
Asem Tengger 500	0,090	0,047	37,68
Asem Tengger 250	0,086	0,040	33,33

From the data obtained, the % results of the stability of the red blood cell membrane from ganjan, muscle flour, perch acid and pangotan. The above results showed that the above plants had the potential for anti-inflammatory activity, therefore it was followed by the IC50 calculation. Table 5 shows the results of the% stability of the red blood cell membrane, from the results obtained, the IC50 value was determined by means of linear regression. The percent stability of the membrane which has a value, perch acid with a positive control of diclofenac sodium. Plants selected by the membrane stability method can be concluded that they have potential as anti-inflammatory, which is then tested by the in vivo method to see its potential as anti-inflammatory. The results of linear regression (%) stability of red blood cell membranes in *Asem Tengger* can be seen in Table 6.

Table 6. Linear regression of red cell membrane stability

	Linear Regression	Results IC_{50}
Asem Tengger	Y= 0,0139x + 30,431	1407 ppm

The stability of human red blood cells can be seen when the red blood cells are induced by a hypotonic solution. This causes the formation of oxidative stress which can disrupt the stability of the biomembrane. Oxidative stress can cause oxidation of lipids and proteins, leading to membrane damage characterized by hemolysis. The size of the hemolysis that occurs in the red blood cell membrane induced by a hypotonic solution is used as a measure to determine anti-inflammatory activity [4].

The results of the IC50 value of the stability of the red blood cell membrane were 1407 ppm in perch acid. IC50 is the concentration which can inhibit 50% of inflammation formation. The IC50 value is often used to test for inhibition of inflammation formation. The smaller the IC50 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 the absorbance of 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 measurements were 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 [12]. Diclofenac sodium at a concentration of 100 ppm can inhibit red blood cell hemolysis by 51%. Another study conducted by Punchard [4] 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 which is widely used to treat inflammation and is easy to obtain.

The results showed that the concentration of 2000 ppm 96% ethanol extract of *Asem Tengger* was able to stabilize the red blood cell membrane by 57.97%. At a concentration of 1000 ppm showed the greatest stabilization ability, namely 44.92%. Whereas at a dose of 500 ppm the smallest stability ability was 37.68% and at a dose of 250 ppm the smallest stability ability was 33.33%. 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 data on the percent stability of red blood cell membranes of diclofenac sodium at a concentration of 100 ppm and plant extracts at concentrations of 250, 500, 1000, 2000 ppm. 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 red blood cell membrane stability data. Diclofenac sodium at a concentration of 100 ppm and plant extracts at a concentration of 250, 500, 1000, 2000 ppm. 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. Percentage 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 [4]. Some references also state the percentage of stabilization as a percentage of inhibition of hemolysis.

2. Results of in vivo plant extract activity testing

a.Test dosage dosage.

Determination of the dose of selected plant extracts given to experimental animals was obtained from previous research doses, namely 75, 150 and 400 mg / g BW of mice [4] after being converted from mice to 200g mice. The results of group and dose determination are presented in table 8 below.

b. Lambda (λ) 1% carrageenan dosage.

The dose of carrageenan used in rats was 0.1 ml / kg BW.

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c. 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.

d. 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 test animals male white rats, 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 8. AUC results and percent anti-inflammatory power of the control solutions and selected plants

Test solution - Sample	dose mg/Kg	AUC ± SD	Percent anti-inflammatory
Negative control (CMC)	-	2.3260 ± 0.1184	-
Positive Control (methyl prednisolone)	0.072	0.3780 ± 0.1188	73,04
Asem tengger	105	1.9532 ± 0.4120 [b]	27,30

Information

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

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

From table 8 above that Asem perch has the% best anti-inflammatory power. The method used in this study was the induction method of edema on the hind legs of test animals using 1% carrageenan. The udema formed will then be measured in thickness using a digital caliper. 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[4]. 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 [4]. 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 [12].

The percentage of anti-inflammatory power data was statistically analyzed to see that there were significant differences in the anti-inflammatory effect 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 (Sujarweni, 2015). 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 In this study, the tamarind plant which has high ICF and UV values but in the in vivo test with the percentage value of inflammatory power is not high because first, it is necessary to use another test method to be able to compare the potential for anti-inflammatory activity, secondly in the Tengger tribe the use of these plants in fresh form, while in the in vivo test using simplicia, it is suspected that some compounds that have the potential to be anti-inflammatory will be damaged during the drying process, thereby reducing the potency of the plant[4].

IV. CONCLUSION

Antimicrobial test of *Asem Tengger* with ethanol 95%, water fraction, ethyl acetate fraction and n hexane fraction. The test results show different activity of each solvent. It was concluded that the tamarind perch tested had potential against Gram-positive, Gram-negative and fungi. Based on the antimicrobial test, *Asem Tengger* has the potential to be antibacterial to Staphylococcus aureus ATCC 2592 in the water and n-hexane fractions with a value of 2.5% MBC. The test results of anti-inflammatory activity IC50 perch acid using the in vitro method had an IC50 value of 1407 ppm. The anti-inflammatory test showed the results of membrane stability of 57.97% from a concentration of 2000 ppm which shows a number that has the potential to be a reference for potential medicinal plants. The *Asem Tengger* plant used by the Tengger tribe has the potential as new antimicrobials and anti-inflammatory

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