ACTIVITY TEST OF METHANOL AND ETHANOL EXTRACT OF REED LEAVES (*Imperata cylindrica L.)* AS AN ANTI-INFLAMMATORY

Wa Ode Helma [1], Sarni [2], Muhammad Tasjiddin Teheni [3]*

[1,2,3]Politekni BauBau, BauBau, Indonesia

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CORRESPONDING AUTHOR

Name : Sarni
Address: Jl. Erlangga Kel. Bone-Bone kota BauBau
E-mail : sarni.230579@gmail.com

A B S T R A C T

The Imperata cylindrica L. It has long been used by Indonesian people as a medicinal plant that has various benefits, including its ability as an anti-inflammatory agent. This plant contains active compounds such as flavonoids, which are believed to have anti-inflammatory effects. The purpose of this study was to evaluate the anti-inflammatory activity of methanol and ethanol extracts of Imperata cylindrica leaves, and to compare the effectiveness of the two extracts. The method used in this study was experimental in the laboratory, with the extraction of Imperata cylindrica leaves using methanol and ethanol solvents. Anti-inflammatory activity was measured in vitro using the UV-Vis spectrophotometry method with bovine serum albumin (BSA) standards at a wavelength of 660 nm. The results showed that the methanol and ethanol extracts of Imperata cylindrica leaves contain compounds that have the potential to have anti-inflammatory activity. Even so, the methanol extract showed weak anti-inflammatory activity with an IC50 value of 194.15 µg/ml. In contrast, the ethanol extract had an IC50 of 209.36 µg/ml, which indicated less significant anti-inflammatory activity. Based on the results of this study, it can be concluded that the extract of cogongrass leaves extracted with methanol has better anti-inflammatory potential compared to ethanol extract, although the effect is still weak. Further research is needed to identify the active compounds responsible for this activity and to improve its therapeutic potential.

INTRODUCTION

In Indonesia, many medicinal plants have not been used optimally for health benefits. Traditional medicine is a cultural heritage that must be preserved and developed to help improve public health (Teheni *et al*., 2023). The use of traditional medicines is considered safe as compared to modern medicine. modern. This is because traditional medicine has fewer side effects than modern medicine (Yolandari & Mustiqawati, 2022). In various ethnic groups in Indonesia, this plant is used for medicine (ethnopharmacology). Various ethnic groups in Indonesia use reeds (Imperata cylindrica L.). Many ethnic groups in Indonesia use the reed plant as a medicine for various diseases, such as allergies, kidney cough, abdominal pain, ulcers, and bleeding. The various local knowledge that the Indonesian ethnic group has about reed plants provides great opportunities for the reed-based herbal medicine sector. A basic element of sustainable development is local knowledge (Manar, 2018).

Reeds (Imperata cylindrica L.) belong to the plant family Gramineae or Poaceae. This plant is generally considered a pest because it grows uncontrollably wherever it is not used. In fact, reeds (Imperata cylindrica L.) offer many advantages. The parts of the reeds that can be used for medicine are the rhizomes and flowers (Kartika et al., 2020).

Reeds are pioneer plants that thrive in the sun where there are flammable parts above the ground and rhizomes that grow widely underground. Due to its strong resistance, reeds force other plants to compete with it in terms of nutrients, water, and sunlight. Because reeds are poisonous plants that can produce allelopathic substances, this plant has an influence that can harm the growth of other plants around it (Yanti *et al*., 2016).

Inflammation is a natural defense reaction against tissue damage caused by chemicals or microorganisms. Inflammation can be temporary or chronic, systematic, and can also occur acutely or that trigger a pathological disorder (Wardani, 2020). These symptoms are characterized by redness, heat, pain, swelling and impaired organ function. Inflammation can be treated using steroids and non-steroidal drugs (AINS) that can relieve inflammation well, but can have side effects if used for a long time.

Based on the results of research on anti-inflammatory activity, that reed extract has ptensi as an anti-inflammatory activity for male rats. And the results of research on leaf and reed root extracts contain allelopathic compounds, namely four groups of phenolic compounds consisting of isofemific acid, salicylic acid, veratatric acid and amic acid as anti-inflammatory (Rahajeng *et al*., 2020).

The use of reed leaves as an anti-inflammatory has been the subject of many previous studies, but the hunt for an effective treatment continues. Besides that, because of the difference in where a plant grows, it will produce different content and effectiveness. For this reason, further research is needed to determine whether reed leaves can effectively reduce inflammation or inflammation by evaluating the anti-inflammatory properties of methanol and ethanol extracts of reed leaves.

METHODOLOGY

This research was conducted in May - June 2024 at the Pharmacy Laboratory of Halu Oleo University (UHO) Kendari. The tools is Blender (philips), stirring rod, test tube rack (pudak), measuring flask (pyrex), beaker (iwaki), sample container (glass jar), vial bottle (glass), vortex (ika), static pole, plactic wrap, clamp, droppipette, spatula, test tube (one med), waterbath (germany), maceration container (glass jar), rotary evaporator (stuart), volume pipette (herma), dropper pipette, measuring cup (iwaki), analytical balance (fujitsu), micro pipette (microlite), Visible spectrophotometer (Labo). The materials in the study were, among others; aquadest, bovine serum albumin (BSA) (sigma), NacCl crystals, glacial acetic acid, FeCl3 1% solution, H2SO4 2N solution, HCl 2N solution, HCl ($_p$) solution, methanol solution (emsure), ethanol solution (emsure), Na0H solution 5%, methyl paraben (nipagin), sodium diclofenac, dragendorff reagent, universal pH, Mg powder, tris base, tris buffer saline (FFB), reed leaf (*Imperata cylindrica L*).

Working Procedure

Sample Preparation

The reed leaves are first cleaned, then cut into small pieces and drained, then aerated at room temperature and not exposed to direct sunlight. Next, it is weighed and mashed using a blender.

Sample Extraction

The extraction process is carried out by the maceration method. Soak the reed leaves with methanol and ethanol solvents in a span of 3x24 hours which is done 1 time. After that, the filtered filtrate is separated from the solvent using a rotary evaporator until a coarse extract of reed leaves is obtained.

Flavonoid Test

The methanol extract of reed leaves is put into a reagent tube as much as 1 mL, then add 3 drops of lauran HCl*(p)*, then add 1 spatula of magnesium powder, stir until evenly distributed and let stand for a while before observing. If there is a change in color to yellow, jinga, red, green or blue, then it indicates the presence of flavonoids

Phenolic Test

A total of 1 mL of methanol extract of reed leaves was put into a test tube, then 5 drops of 1% FeCl3 solution were added, and homogenized, then let stand for a while and then observed. Positive presence of phenolic content which is characterized by color changes or reactions, namely green, blue, red, black or deep purple.

Steroid Tests

Add a few drops of *Lieberman-buchard* reagent to 1 mL of methanol extract of reed leaves then stir homogeneously and observe. *The Lieberman-buchard* reagent is 5 drops of anhydrous acetic acid solution and 2 drops of H2SO4 solution (p). A positive result for steroids is marked by the appearance of a blue or green ring.

Manufacture of Tris Buffer Saline (FFB) Solution

Tris Buffer Saline (FFB) or often referred to as Tris buffer solution, is a solution used in a variety of laboratory applications, especially in biochemistry and molecular biology. TBS is commonly used in protein analysis, electrophoresis, immunooblotting, and antigen-antibody reactions.

NaCI was put into a 500 mL measuring flask of 4.35 grams, 200 mL of aquadest was added along with 0.605 grams of tris base. The solution is shaken and then another 200 mL of aquadest is added. The pH of the solution is regulated by adding glacial acetic acid until it reaches pH 6.3 then aquadest is added until it reaches the specified volume limit to the specified limit and stirred until homogeneous.

Preparation of Bovine Serum Albumin (BSA) Solution in Tris Buffer Saline (FFB)

Bovine Serum Albumin (BSA) or often referred to as porcine serum albumin is a protein compound that has various applications in the fields of biology, pharmaceuticals, and the food industry (Sarni *et al*., 2023). BSA as much as 0.2 grams, added a little aquades, homogenized then put into a 100 mL measuring flask, then added FFB to the limit of the stamping and then beaten until homogeneous.

Negative Control Solution Manufacturing

50 µL of methanol is put into a 5 mL measuring flask, then 0.2% BSA solution is added to the mark, and beaten until homogeneous.

Positive Control Solution Formulation

A total of 0.025 g of diclofenac sodium was weighed and put into a 25 mL measuring flask, then dissolved with aquadest until it reached the limit mark and stirred until homogeneous, so that a parent solution with a concentration of 1000 ppm was obtained. The parent solution of sodium diclofenac (1000 ppm) was then diluted using methanol solvent to produce a variation in sodium diclofenac concentration of 25; 50; 100; 200 and 12.5 ppm.

Anti-Inflammatory Activity Test

50 mL of positive control solution and 50 mL of test solution were each taken using a micropipette, then transferred into a 10 mL measuring flask, then add 0.2% BSA to 10 mL. then put the solution into an incubator with a temperature of about 25oC and leave for 30 minutes, after which the solution was heated using *a water batch* at a temperature of about 72 ^{oC} for 5 minutes. After heating, the solution is left at room temperature for about 25 minutes, after cooling the positive control solution and the test solution are homogenized using a vortex. Then the absorption was measured using a visible spectrophotometer at a wavelength of 660 mm (Saleh *et al*., 2023).

Data Analysis

The data analysis carried out in this study used a linear regression equation calculated from the BSA standard solution to determine the % inhibition or percentage of denaturation inhibition against inflammation in the following equation.

$$\text{Inhibition \%} = \frac{\textit{Negative control absorbance - Absorbance of test solution}}{\textit{Negative control absorbance}} \quad 100$$

If the % of inhibition is more than 20% then the sample is suspected of having anti-inflammatory activity, while the IC50 value is calculated based on the linear regression equation of concentration (x) to % of inhibition (Y).

RESULTS & DISCUSSION

Results of Reed Leaf Extract (Imperata cylindrica L)

Table 1. Results of Reed Leaf Extract (*Imperata cylindrica L*)

Sample	Solvent	Heavy Initial sample	Final Sample Weight	Yield Results
Reed leaves	Methanol	250 grams	3.99 grams	1,6%
	Ethanol	250 grams	2.99 grams	1,2%

Based on the results of extraction using methanol solvent and the weight of simplicia was 250 grams and the final sample weight was 3.99 grams, the percentage of extract yield was obtained which was 1.6%. Meanwhile, the results of extraction using ethanol with an initial sample weight of 250 grams and a final sample weight of 2.99 grams were obtained with a percentage of extract yield of 1.2%.

Table 2. Flavonoid test results of ethanol and methanol extracts of reed leaves (*Imperata cylindrica L*)

Sample Name	Kind Testing	Reagent Type	Change Color/Reaction	Result
Ethanol Extract Leaf Reeds	Flavonoids	Concentrated Mg + HCl Powder	Yellowish	+
Methanol Extract Reeds	Flavonoids	Concentrated Mg + HCl Powder	Redness	+

Source : Primary Data, 2024

Caption : + = Positive

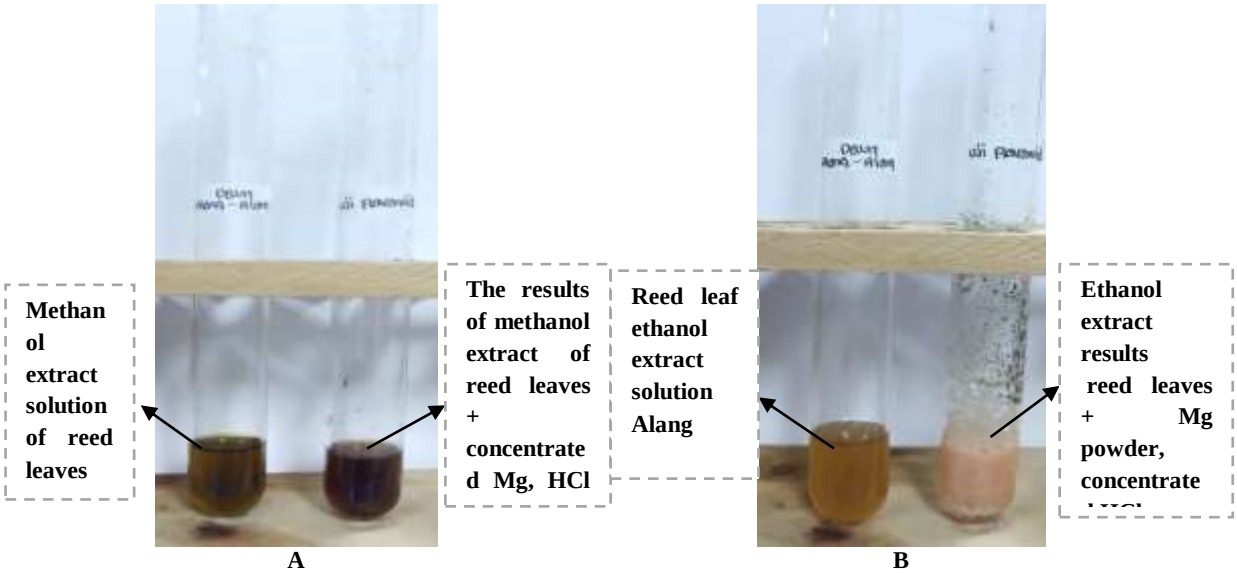

Figure 1. Flavonoid Screening Test Results for Methanol (A) and Ethanol (B) Extract of Reed Leaves (*Imperata cylindrica L*)

Table 3. Results of Phenolic Test of Ethanol and Methanol Extract of Reed Leaves (*Imperata cylindrica L*)

Sample Name	Kind Testing	Reagent Type	Change Color/Reaction	Result
Ethanol Extract Reeds	Phenolic	FeCI3 5%	Black	+
Methanol Extract Reeds	Phenolic	FeCI3 5%	Black	+

Source : Primary Data (2024)

Caption : + = Positive

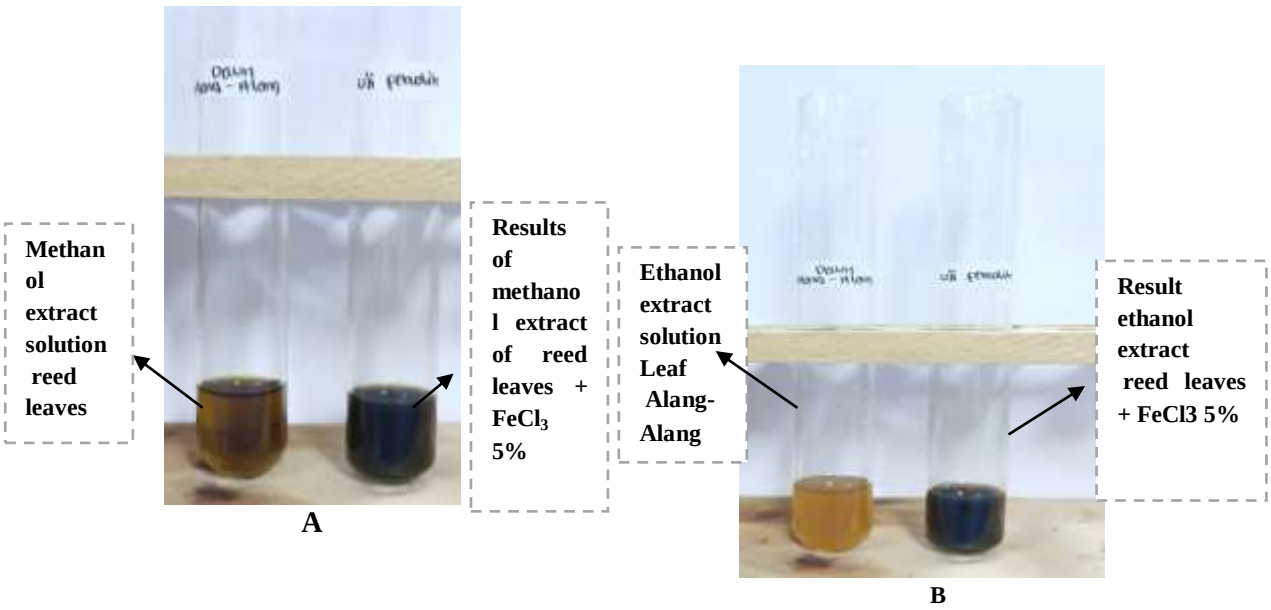

Figure 2. Results of phenolic screening test for methanol (A) and ethanol (B) extract of reed leaves (*Imperata cylindrica L*)

Table 4. Steroid Test Results of Ethanol and Methanol Extract of Reed Leaves (*Imperata cylindrica L*)

Sample Name	Kind Testing	Reagent Type	Change Color/Reaction	Result
Ethanol Extract Reeds	Steroids	*Lieberman Burchard*	No Color Change	-
Methanol Extract Reeds	Steroids	*Lieberman Burchard*	No Color Change	-

Source : Primary Data, 2024

Caption : + = Negative

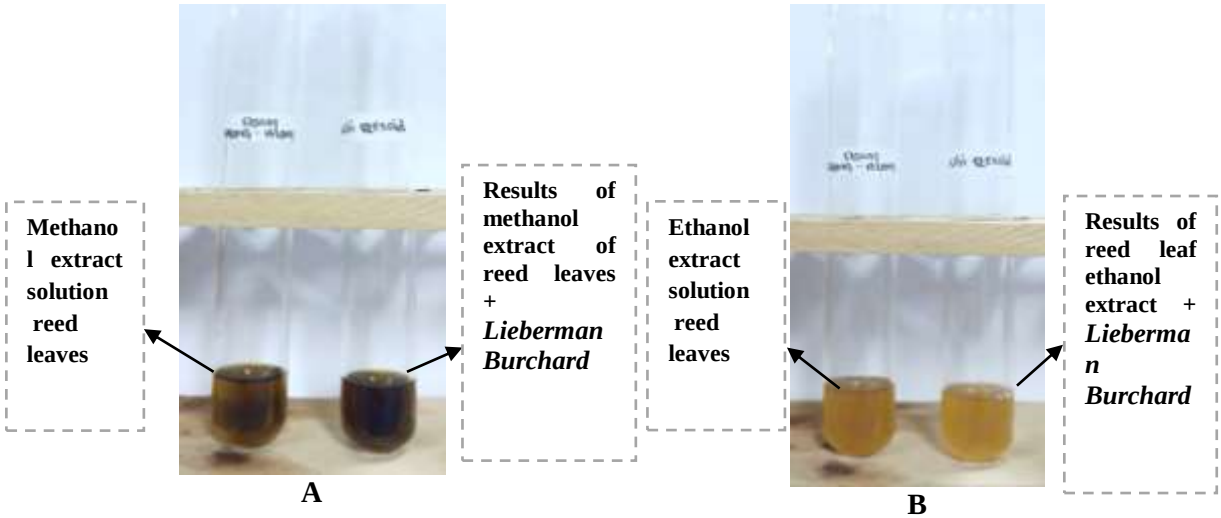

Figure 3. Steroid screening test results for methanol (A) and ethanol (B) extract of reed leaves (*Imperata clindrica L*)

Anti-Inflammatory Activity Test Results of Methanol Extract of Reed Leaves *Impera cylindrica L*)

The results of the measurement of % inhibition of methanol extract of reed leaves using UV-Vis at a wavelength (**λ**) of 660 nm.

Table 5. Anti-Inflammatory Activity Test Results Measurement of Methanol Extract of Reed Leaves Using UV-Vis or Wavelength (λ) 660 nm.

Concentration (mg/L)	%Inhibisis			Average	Equation Regression	IC50 (µg/mL)
	U1	U2	U3			
31,25	35,25	35,16	35,25	35,22		
62,5	35,80	35,71	35,80	35,77		
125	41,70	41,62	41,70	41,67	y= 0.0913x + 30.867	194,12
250	53,22	53,16	53,22	53,20	R2 = 0.997	
500	76,95	76,92	76,94	76,94		

Source : Primary Data, 2024

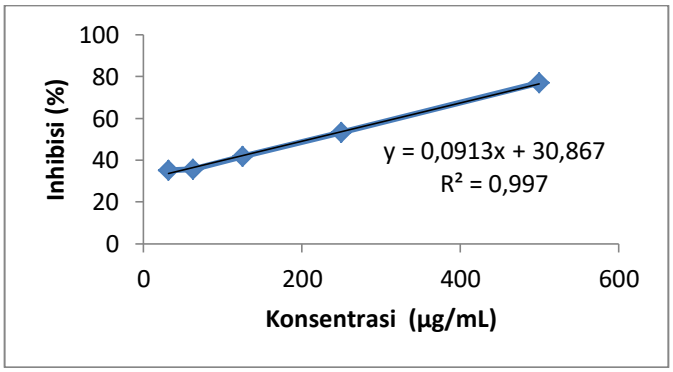

Figure 4. Results Of Convergence Linear Regression Curve And % Inhibition Of Methanol Extract Of Reed Leaves

Anti-Inflammatory Activity Test Results of Ethanol Extract of Reed Leaves (*Imerata cylindrica L*)

The results of the measurement of % inhibition of methanol extract of reed leaves using UV-Vis at a wavelength (**λ**) of 660 nm.

Table 6. Anti-Inflammatory Activity Test Results Measurement of Ethanol Extract of Reed Leaves Using UV-Vis or Wavelength (λ) 660 nm.

Concentration (mg/L)	%Inhibisis			Average	Equation Regression	IC50 (µg/mL)
	U1	U2	U3			
31,25	35,25	35,16	35,25	35,22		
62,5	35,80	35,71	35,80	35,77		
125	41,70	41,62	41,70	41,67	y= 0.0913x + 30.867	209,56
250	53,22	53,16	53,22	53,20	R2 = 0.997	
500	76,95	76,92	76,94	76,94		

Source : Primary Data, 2024

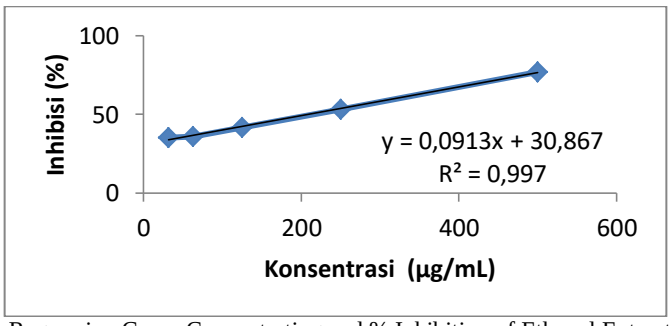

Figure 5. Linear Regression Curve Concentrating and % Inhibition of Ethanol Extract of Reed Leaves

Test Results of Anti-inflammatory Activity of Sodium Diclophonene

The results of the measurement of % Inhibis of methanol extract of reed leaves using UV-Vis at a wavelength (**λ**) of 660 nm.

Table 7. Test Results of % Inhibition of Positive Control of Sodium Diclofenac

Concentration (mg/L)	%Inhibisis			Average	Equation Regression	IC50 (µg/mL)
	U1	U2	U3			
31,25	10,97	10,85	10,97	10,93		
62,5	43,90	43,90	43,90	43,87		
125	68,04	68,99	68,04	68,02	y= 0.3619x + 32.587	48,1
250	78,88	78,85	78,88	78,87		
500	96,00	96,00	96,00	96,00		

Source : Primary Data, 2024

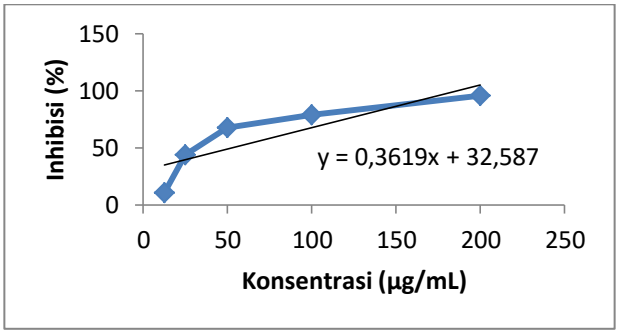

Figure 6. Results of Linear Regression Curve Concentration and % Inhibition of Diclophenac Sodium

Discussion

The sample in the study was reed leaves (*Imperata cylindrica L.*). As much as 3.5 kg was taken, the reed leaves were sorted wet and then washed with running water. Stretching, then drying in an open place without direct sunlight. This is done to reduce the risk of damage to chemical components on reed leaves due to exposure to high temperatures in the sun. The dried reed leaves are then sorted dry to separate the small stems from the reed leaves. The reed leaves that have been sorted are then blended until they become 500 grams of reed leaf powder simplicia, then divided into two reed leaf simplises of 250 grams for ethanol sailors and 250 grams for methanol solvents, then extraction is carried out by the maceration method using ethanol and methanol solvents as much as 2 L for 3 x 24 hours with one mixing.

In this study, extraction was carried out using the maceration method. The maceration method is the process of extracting active substances from solid materials such as plants using solvents. During the maceration process, the solvents used are 96% ethanol and 2 L methanol (Dewatisari, 2020). After maceration, filtration is carried out to separate the filtrate from the residue. After that, the filtrate that has been obtained is then rotated by an evapotaror with a temperature of 65o to produce a thick extract of reed leaves where methanol solvent is 27.29 grams of methanol with a yield of 1.6% and ethanol extract of 20.53 grams with a yield of 1.2%. In the previous study, the yield value of reed leaf extract obtained was 1.6% methanol extract and 1.2% ethanol extract. The higher the yield, the greater the number of active compounds contained in the sample (Saputri et al., 2022). The higher the yield value, the larger the extract produced, the more efficacious substances obtained contained in the reed leaves.

The difference in levels obtained from reed leaves (*Imperata cylindrica L*) is influenced by the environment in which the plant grows. This environmental influence is closely related to the plant metabolic process, namely the biochemical process and the synthesis of secondary metabolite compounds. Environmental factors such as the altitude of places with high environmental stress, such as high temperature, high humidity and low sunlight intensity can affect the production of secondary metabolites including flavonoid and antioxidant levels in plant extracts (Sarni *et al*., 2020).

This extract will be used to conduct qualitative tests with phytochyma screening which is one of the ones carried out to identify the content of secondary metabolite compounds of a plant based on the color produced from the reaction using certain reagents. Furthermore, an anti-inflammatory activity test of reed leaf extract produced in vitro using bovine serum albumin (BSA) was carried out. The use of BSA solution is necessary because BSA is considered a more sensitive protein denaturation biomarker than other albumin, so BSA was chosen in this study.

The solvents used are ethanol and methanol, namely to extract or withdraw compounds that have the potential to be anti-inflammatory in this study are ethanol and methanol. The selection of these two solvents is due to the fact that some secondary metabolite compounds are semi-polar and some are non-polar, so they must be dissolved with semi-polar solvents that can attract polar compounds and non-polar compounds. The selection of these two solvents is to determine the factors or effects of the solvents on the anti-inflammatory activity of these reed leaf extracts.

The results of the phytochemical screening test of flavonoid compounds in ethanol extract solution and reed leaf methanol in Table 1, it can be seen that both extracts are positive for flavonoid compounds. This can be seen in the color change in the ethanol extract of reed leaves where there is a change in the color of the green extract to orange. As for the methanol reed leaf extract, it shows a red color after adding specific reagents for flavonoids. Positive for flavonoid content if the solution turns yellow, orange, red, green to blue (Erwin *et al*., 2020).

The phytochemical screening test of phenolic compounds in ethanol extract solution and reed leaf methanol in Table 2, showed positive that both extracts contained phenolic compounds. This can be seen in the color change in the ethanol extract of reed leaves where there is a change in the color of green excrement to black. As for the methanol reed leaf extract, it shows a black color. After adding specific reagents for phenolics, if positive it contains ocean phenolic changes color to green, black, blue, red or deep purple (Maulina *et al.*, 2019). The results of the phytochemical screening test of steroid compounds, ethanol extract solution and methanol of reed leaves were negative for steroid compounds. It can be seen that there is no color change in the ethanol extract of reed leaves and methanol extract of reed leaves. Once the specific reagent for the steroid is added, if it is positive for the steroid content the solution changes to green and blue (Erwin *et al*., 2020).

Based on the results of phytochemical screening of steroid compound tests of ethanol extract solution and reed leaf methanol in Table 3, it can be seen that both extracts are negative for steroid compounds. It can be seen that there is no color change in the ethanol extract of reed leaves and methanol extract of reed leaves. After adding specific reagents for steroids, if it is positive for steroids the solution changes to green and blue colors (Erwin E *et al*., 2020).

An in vitro anti-inflammatory test was conducted to evaluate the ability of methanol and ethanol extracts from reed leaves to inhibit protein denaturation. Protein is one of the triggers for inflammation or inflammation. The protein, which in this case is Bovine serum albumin, is a trigger for inflammation or inflammation. Protein denaturation is induced by heat resulting in denaturation, which is caused by successive heating and denaturation of chemicals. Based on the results of the calculation of the methanol exract, the sample was declared to have very strong anti-inflammatory activity if the IC50 value < 50 ppm, strong if the IC50 value was 50-100, while if the IC50 value was 100-150 ppm, weak if the IC50 value was 151-200 ppm, it was declared inactive if it had an IC50>200 value (Mulyani *et al.*, 2023).

The results of the anti-inflammatory activity test of methanol extract of reed leaves measured using UV-Vis at a wavelength (λ) of 660 nm in Table 5. It is known that at the Concentration variation of 31.25; 62; 5 125; 250 and 500 methanol extracts of reed leaves (*Imperata cylindrica L*) can inhibit denaturation so that it can be categorized as potentially anti-inflammatory although weak. The lowest protein inhibition denaturation was at a concentration of 31.25 with an inhibition of 35.64 and at a concentration of 500 was a high inhibition value with an inhibition value of 79.00. Meanwhile, the results of the anti-inflammatory activity test of ethanol extract of reed leaves using UV-Vis at a wavelength (λ) of 660 nm, in Table 6. It is known that at a concentration variation of 31.25; 62; 5 125; 250 and 500 ethanol extracts of reed leaves (*Imperata cylindrica L*) could not inhibit denaturation so they were categorized as not potentially anti-inflammatory, protein inhibition denaturation was the lowest at a concentration of 31.25 with an inhibition of 35.22 and the highest inhibition was at a concentration of 500 with an inhibition value of 76.94. The IC50 calculation for the antiinflammatory activity of methanol extract using the linear regression equation (y=0.0926x + 32.0219) of 194.15 ppm is indicated to be weak and the IC50 of ethanol extract with the liner regression equation (y=0.0913x + 30.867) of 209.56 ppm does not have anti-inflammatory.

This difference in anti-inflammatory activity is related to the solvents used in the extraction, namely methanol and ethanol of reed leaves *(Imperata cylidrinca L).* From the results, it can be seen that the methanol extract has anti-inflammatory activity although it is weak. This indicates that the secondary metabolite that has anti-inflammatory activity is well extracted with methanol solvents even if it is only a small amount. Meanwhile, reed leaf extract with ethanol solvent does not provide anti-

inflammatory activity. This indicates that secondary metabolite compounds are not well interested in this very weak use of ethanol solvents.

Based on previous research on this reed extract, especially reed stem ethanol extract, it is stated that reed leaf stem extract (*Imperata cylindrica L*) has the greatest anti-inflammatory effect in male rats (Vika *et al.,* 2020). This proves that this reed leaf extract contains very few or very little secondary metabolic compounds that are active as anti-inflammatory compared to reed stem extract. Based on previous research on this reed extract, especially reed stem ethanol extract, it is stated that reed leaf stem extract (*Imperata cylindrica L*) has the greatest anti-inflammatory effect in male rats (Vika *et al.,* 2020).

CONCLUSION

From the results of the research that has been carried out, it can be concluded that the crude extract of reed leaves *(Imperata cylindrica L*) contains flavonoid and phenolic compounds and has anti-inflammatory activities, especially methanol extract and crude methanol extract of reed leaves as anti-inflammatory activities *(Imperata cylindrica L)* had weak anti-inflammatory activity with an IC50 of 194.15µg/mL and crude ethanol extract of reed leaves with an$_{IC50}$ of 209.56 µg/mL (*Imperata cylindrica L*) had no anti-inflammatory activity.

The limitations in this study need to be considered. The first is environmental variability, environmental conditions where plants grow, such as altitude, temperature, humidity and sunlight intensity, can affect the levels of flavonoids and phenolics and anti-inflammatory activity in reed leaves. Since the study included only one village in the southeastern Sulawesi city of Baubau, the results may not fully represent a wider variation in other environmental conditions.

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