Test of Anti-Inflammatory Activity of 70% Ethanol Extract of Binahong Leaves (Anredera cordifolia) in Male White Rats (Rattus norvegicus) Induced with Carrageenan

Marsanda Dara Paquita[1]*, Wima Anggitasari[2], Dhina Ayu Susanti[3]
[1.]Bachelor of Pharmacy, Faculty of Health Sciences, Universitas dr. Soebandi, 68111
[2.]Bachelor of Pharmacy, Faculty of Health Sciences, Universitas dr. Soebandi, 68111
[3.]Bachelor of Pharmacy, Faculty of Health Sciences, Universitas dr. Soebandi, 68111
Email: marsandadara29@gmail.com

ABSTRACT

Inflammation is a protective response to tissue damage due to physical trauma. Steroid treatment can reduce prostaglandin release but often increases the risk of diabetes as a side effect. NSAIDs can address inflammation by inhibiting COX-1 and COX-2 enzymes, but may cause gastrointestinal damage. Therefore, there is a need for traditional and natural medicine alternatives that can stabilize free radicals and prevent cell damage, such as the binahong plant. This plant contains flavonoid compounds with high antioxidant properties, which can neutralize free radicals.This study aims to determine the anti-inflammatory activity of 70% ethanol extract of binahong leaves (Anredera cordifolia) in male white rats (Rattus norvegicus) induced with carrageenan. This study employs a laboratory experimental design with 20 test animals divided into 4 treatment groups, with extraction performed using the maceration method. In the screening test, a tube method is used, and anti-inflammatory testing is conducted by observing changes in the volume of rat paws before and after carrageenan injection. Data processing and analysis are performed using ANOVA in SPSS software. The percentage of edema was found to be 7.41% for the 200 mg/kg BB dose, 6.98% for the 400 mg/kg BB dose, 7.60% for the positive control, and 19.76% for the negative control. Normality and homogeneity tests showed normal and homogeneous results. ANOVA results indicated no significant differences, while LSD tests showed significant differences between the negative control and all treatment groups. Screening results (+) indicated the presence of flavonoid compounds.The ethanol extract of binahong leaves has anti-inflammatory activity as evidenced by the percentage of edema (7.41% and 6.98%) in male white rats induced with carrageenan. The most effective extract dosage is 200 mg/kg BB.

Keywords: Anti-inflammatory, Binahong Leaves, Carrageenan, Anredera cordifolia

INTRODUCTION

Inflammation is a local protective response that occurs due to tissue damage caused by physical trauma, harmful chemicals, or microbiological pathogens. The purpose of inflammation is to destroy, reduce, or limit both the damaging agents and the damaged tissue. Symptoms of inflammation include swelling, redness, heat, pain, and changes in function (Ramadhani dan Sumiwi, 2016). Inflammation can be an indicator of disease, including degenerative diseases common in older age, such as Rheumatoid Arthritis (RA). RA is an autoimmune disease that affects various tissues, primarily the joints, and can also impact other organs such as blood vessels, skin, eyes, and lungs (Savitri *et al*., 2019). According to the World Health Organization (WHO), approximately 20% of the global population is affected by RA, with a prevalence of 5-10% in individuals aged 5-20 years and 20% in those aged 55 years. In 2020, there were 355 million RA patients worldwide, and this number is expected to increase by more than 25% by 2025, with a potential for paralysis (Suswitha dan Arindari, 2020). In Indonesia, the elderly

population continues to grow, with data from the Central Statistics Agency showing a 10.82% increase over the past 50 years, reaching 29.3 million people. The prevalence of RA in East Java is 10.8% (Adhari, Suryawati, dan Gani, 2023), and data from the Health Office indicates that 22.3% of the elderly in Jember Regency suffer from joint diseases (Hanso, 2016).

Treatment for RA aims to reduce pain, swelling, and stiffness through both non-pharmacological and pharmacological therapies. Pharmacological therapy involves the use of low-dose anti-inflammatory drugs to relieve symptoms. Anti-inflammatory drugs are divided into two groups: steroids and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) (Cruijssen 2020). NSAIDs work by inhibiting cyclooxygenase-1 and 2 (COX-1 and COX-2) enzymes, thereby reducing the production of prostaglandins and prostacyclins, which are mediators of inflammation. This leads to vasoconstriction and inhibits prostaglandin production, which can increase sodium retention. The use of NSAIDs can lead to complications such as hypertension, edema, kidney dysfunction, and gastrointestinal bleeding (Imananta dan Sulistiyaningsih, 2018).

In Indonesia, traditional medicine is still used by many people for various ailments. Traditional medicine, or herbal medicine, uses natural ingredients (Adiyasa dan Meiyanti, 2021).One plant with potential anti-inflammatory properties is binahong, which belongs to the Basellaceae family and is empirically considered to have medicinal potential (Hutauruk, Rosita, dan Oktavianawati, 2014). Research shows that binahon has anti-inflammatory properties due to its content of compounds such as saponins, flavonoids, and high water content. Flavonoids, as antioxidants, help neutralize and stabilize free radicals, preventing damage to healthy cells and tissues and regulating oxidation processes in the body (Anwar dan Soleha, 2016).

MATERIAL AND METHODS

Determination

The determination of binahong leaves (Anredera cordifolia) was carried out to identify the plant species and confirm the authenticity of the material. The plant determination was done at the Laboratory of Politeknik Jember.

Research Ethics

The procedures for the anti-inflammatory activity test on male white rats were ethically approved by the Chairperson of the Ethics Committee at Universitas dr. Soebandi Jember with No. 294/KEPK/UDS/IV/2024, confirming the ethical validity of the research.

Research Time and Place

This study was conducted from May to July 2024 at the Integrated Laboratory of Universitas dr. Soebandi Jember.

Equipment and Materials

The equipment used in this study included rat cages, feeding and drinking containers, animal scales, clocks, plethysmometer, 1cc syringes, beakers (Pyrex), analytical scales, stirring rods, markers, mortar and pestle, measuring cylinders (Pyrex), blenders, 20 mesh sieves, test tubes, sondes, and rotary vacuum evaporator.

The materials used included binahong leaves, 70% ethanol, 1% carrageenan, diclofenac sodium tablets, 0.5% CMC Na, male white rats, NaCl, concentrated HCl, and magnesium (for screening).

Preparation of Binahong Leaf Ethanol Extract

The extract preparation used binahong leaves (Anredera cordifolia) obtained from Pancor

Village, Sumenep Regency, East Java. The collected samples were sorted, washed, choppe s a significant difference, $p < 0.05$.

dried under sunlight, ground, and sieved using a 20 mesh sieve. The obtained binahong leaf powder was weighed at 300 grams and macerated with 1.5 liters of 70% ethanol, covered with aluminum foil. The maceration process continued with remaceration for 3x24 hours, with a total solvent volume of 4.5 liters. The soaked material was filtered through filter paper, and the filtrate was concentrated using a rotary evaporator at 50°C.

Preparation of 1% CMC Na Suspension

0.50 grams of CMC Na was weighed and gradually added to 100 ml of heated distilled water while stirring until homogeneous. It was then transferred to a storage container.

Preparation of Sodium Diclofenac Suspension

10 diclofenac sodium tablets were weighed, and the average weight of all tablets was calculated before grinding them into a fine powder. The powdered diclofenac sodium was mixed with 0.5% CMC Na suspending agent to a total volume of 100 ml.

Preparation of Binahong Leaf Ethanol Extract Suspension

0.2 grams and 0.4 grams of concentrated binahong leaf extract were weighed and suspended in 50 ml of CMC Na solution.

Preparation of Carrageenan Suspension

1 gram of carrageenan was weighed and added to 10 ml of NaCl.

Treatment of Test Animals

Twenty male white rats were divided into 5 groups, with 4 rats per group, aged 2-3 months. The rats were adapted for 7 days and fasted for 8 hours before treatment. The volume of each rat's paw was measured to obtain the initial paw volume (V0). Each rat was then injected with 0.1 ml of carrageenan intraplantarly and allowed to rest for 60 minutes. After 60 minutes, the paw volume was measured again to obtain the edema volume after carrageenan induction (Vt). The rats were then treated according to their group, and edema was measured every 60 minutes for 360 minutes.

Data Processing and Analysis

The data obtained were edema volumes, which were calculated to get the percentage of edema. The data were analyzed using One-Way ANOVA with SPSS software. SPSS was used to determine differences in anti-inflammatory activity among treatment groups. One-Way ANOVA began with Shapiro-Wilk normality test and Levene's homogeneity test, which showed normal and homogeneous results. After meeting the normality and homogeneity criteria, One-Way ANOVA was conducted with a 95% confidence level and $p<0.05$. Post hoc tests were then performed using Least Significant Difference (LSD) for further comparison.

RESULT AND DISCUSSION

Plant Determination

The determination of this plant was conducted at the Jember Polytechnic Laboratory, with certificate number: 53/PL17.8/PG.2024. The results indicate that the specimen is Anredera cordifolia (Binahong).

Extraction

The Binahong leaf extract produced in this study is dark green, has the distinctive aroma of Binahong leaves, and has a thick texture. The plant material was extracted using 70% ethanol via maceration and then concentrated using a rotary evaporator. Previous research used 96% ethanol for extraction, resulting in a dark brown extract with a distinctive Binahong aroma and a thick texture. The difference between the two methods is that higher ethanol concentrations can dissolve more components from the plant material.

In this study, the extraction yield was 11.66% from a thick extract weighing 35 grams. Yield refers to the proportion of metabolites obtained after extraction relative to the weight of the sample used. A yield above 10% is considered good (Wardaningrum, 2020). The extraction used 70% ethanol with 300 grams of plant material, producing 35 grams of thick extract with a yield of 11.66%. Previous studies reported a yield of 15.63% (Tjahjani dan Lestari, 2022) using 70% ethanol and maceration. Variations in yield can be influenced by the amount of solvent and extraction temperature. More solvent generally extracts more components from the plant material.

Phytochemical Screening

Phytochemical screening was performed using a test tube method to identify the compounds present in the Binahong leaf extract.

The phytochemical screening revealed that the ethanol extract of Binahong leaves contains flavonoids. Flavonoids are soluble in organic solvents like 70% ethanol, which is an effective solvent for extracting polyphenolic compounds due to its polarity and ability to form hydrogen bonds with ethanol. Previous studies (Cut Bidara Panita Umar, Amelia Niwelle, dan Syntia Clarce Ririmasse, 2023) also reported that Binahong leaf ethanol extract contains flavonoids. These flavonoids are used for anti-inflammatory treatment by inhibiting cyclooxygenase and lipoxygenase activities, thus blocking the biosynthesis of prostaglandins and leukotrienes. The inhibition by flavonoids can accelerate the reduction of inflammation or speed up healing.

Anti-inflammatory Activity Test in Male White Rats

The anti-inflammatory activity in male white rats was measured using a mercury plethysmometer. The test subjects were male white rats (Rattus norvegicus) aged 2-3 months. Male rats were chosen for stability in results, as they are not affected by menstrual cycles or pregnancy, unlike female rats. Male rats also have a faster drug metabolism and more stable biological conditions compared to females (Setya Enti Rikomah, 2019). Prior to the anti-inflammatory test, the rats were adapted for 7 days to facilitate observation, handling, and health monitoring. During adaptation, the rats were kept in clean, adequately-sized cages with sufficient food and water. They were then fasted for 8 hours (fasting only before treatment) to ensure that the active compounds in the Binahong leaf extract were not affected by food, preventing interference in the anti-inflammatory activity test.

Edema measurement was conducted according to the treatment group distribution. The treatment groups included 4 groups: positive control (sodium diclofenac), negative control (CMC Na), Binahong leaf extract at doses of 200 mg/kg BW, and 400 mg/kg BW. Edema measurements were taken 8 times: the first measurement was the baseline (V0), the second after administering carrageenan injection (Vt), and subsequent measurements at hours 1, 2, 3, 4, 5, and 6. After obtaining V0, Vt, and data from hours 1-6, the edema volume and percentage of edema were calculated based on the degree of swelling caused by carrageenan induction

The positive control group had an edema percentage of 7.60 ± 2.13, while the negative control group had a significantly higher percentage of 19.76 ± 4.06. The percentage of edema from the 200 mg/kg BW dose was higher than that from the 400 mg/kg BW dose. Statistical analysis showed significant differences between the negative control group and the positive

control, and between the 200 and 400 mg/kg BW treatment groups ($p < 0.05$). This indicates a reduction in edema; however, CMC Na did not exhibit anti-inflammatory activity as it is neutral and only acts as a solvent. The positive control (sodium diclofenac) showed no significant difference from the 200 and 400 mg/kg BW treatment groups ($p > 0.05$), indicating that sodium diclofenac, an NSAID with high anti-inflammatory potential, effectively reduced edema by inhibiting COX-1 and COX-2 enzymes.

The 200 mg/kg BW dose showed no significant difference from the 400 mg/kg BW dose ($p > 0.05$). This suggests that the ethanol extract of Binahong leaves has anti-inflammatory effects comparable to sodium diclofenac. The extract contains flavonoids, which can reduce edema due to inflammatory responses.The average edema percentage for the 200 mg/kg BW dose was 7.41 ± 2.38, and for the 400 mg/kg BW dose, it was 6.98 ± 2.20, making the effective dose in this study 200 mg/kg BW.

CONCLUSION

The results of this study conclude that 70% ethanol extract of binahong leaves exhibits anti-inflammatory activity based on the percentage of edema at doses of 200 mg/kg BW and 400 mg/kg BW, which are 7.41% and 6.98%, respectively. The effective dose in this study is 200 mg/kg BW.

ACKNOWLEDGEMENT

Gratitude is expressed to God Almighty for His blessings, which made the completion of this research possible. Thanks are also due to everyone who contributed to this research, including parents, siblings, mentors, and friends.

REFERENCES

Ramadhani N, Sumiwi SA. (2016). Anti-inflammatory Activity of Various Plants Suspected to Contain Flavonoids. J Farmaka; 14(2):111–23.

Savitri SA, Kartidjo P, Rahmadi AR, Vikasari SN. (2019). Relationship Between Drug Selection and Therapy Success in Rheumatoid Arthritis Patients. Indones J Clin Pharm; 8(4):237.

Suswitha D, Arindari DR. (2020). The Relationship Between Physical Activity and Rheumatoid Arthritis Pain in the Elderly at a Social Welfare Center. J 'Aisyiyah Med; 5(2):120–30.

Adhari S, Suryawati I, Gani A. (2023). Rheumatoid Arthritis Pain and Independence in Activities of Daily Living (ADL) in the Elderly. J Assyifa' Ilmu Kesehat; 8(2):61–72.

Hanso B. (2016). The Effect of Rheumatic Exercise on Foot Joint Pain in the Elderly at Nusa Indah 43 Elderly Posyandu, Karang Anyar Village, Ambulu District, Jember Regency. Muhammadiyah Univ Jember; 4:1–23.

Cruijssen F. (2020). Literature Review. Int Ser Oper Res Manag Sci; 297(1):49–66.

Imananta FP, Sulistiyaningsih. (2018). Review Article: The Use of NSAIDs (Non-Steroidal Anti-inflammatory Drugs) Inducing Increased Blood Pressure in Arthritis Patients. J Farmaka; 16(1):72–9.

Adiyasa MR, Meiyanti M. (2021). Utilization of Traditional Medicine in Indonesia: Distribution and Demographic Factors Affecting It. J Biomedika dan Kesehat; 4(3):130–8.

Dewi SK, Fikri AA. (2021). Analysis of Binahong and Its Utilization in Coastal Communities of Pantura. NECTAR J Pendidik Biol; 2(2):1–7.

Anwar TM, Soleha TU. (2016). Benefits of Binahong Leaves (Anredera cordifolia) as Therapy for Acne Vulgaris. Univ Lampung; 5(5):179–83.

Wardaningrum. (2020). Plant Determination in Purple Sweet Potato Flesh Extract. Thesis; 1–8.

Tjahjani NP, Lestari DW. (2022). The Potential of 70% Ethanol Extract of Binahong Leaves (Anredera Cordifolia (Ten.) Steenis.) and 96% Ethanol Extract of Green Betel Leaves (Piper Betle L.) Against Proteus mirabilis Bacteria. J Pranata Biomedika; 1(1):64–77.

Cut Bidara Panita Umar, Amelia Niwelle, Syntia Clarce Ririmasse. (2023). Anti-bacterial Activity Test of Ethanol Extract of Binahong Leaves (Anredera Cordifolia (Ten.) Steenis) Against Staphylococcus Epidermidis Bacteria. J Pengabdi Ilmu Kesehat; 2(2):78–85.

Setya Enti Rikomah DM. (2019). Effectiveness Test of Anti-inflammatory Extract of Kepok Banana Peel (Musa acuminate x balbisiana'saga') on Male White Mice (Mus musculus). J Teknol dan Sist Inf Univrab [Internet]; 1(1):2019.

Rasyid I, Hasan T, Noer SF, Bariun H. (2016). Anti-inflammatory Test of Ethanol Extract of Binahong Leaves (Anredera cordifolia Ten) on White Rats (Rattus norvegicus) Induced with 1% Albumin. 4:23–7.

Table 1. Yield Calculation

TABLE AND FIGURE

Sampel	Dry Sample We ight (g)	Thick Extract We ight (g)	Yield (%)
Ekstrak daun binahong	300 gram	35 gram	11,66%

Table 2. Phytochemical Screening

Compound	Reagent	Change Observed	Description
Flavonoid	Mg + Concentrated HCl		

Ta ble 3. An ti -in fla mma tory Ac t iv it y of E thanol Extract of Binahong Leaves

Group	Percentage of Edema (%) ± SD
Negative Control (CMC Na 0.5%)	19,76 ± 4,06*
Positive Control (Sodium Diclofenac)	7,60 ± 2,13
Binahong Leaf Extract 200 mg/kg BB	7,41 ± 2,38
Binahong Leaf Extract 400 mg/kg BB	6,98 ± 2,20

Note: (*) indicates a significant difference, p < 0.05