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In Vitro Antioxidant and Anti-Inflammatory Properties of Ethyl Acetate Extract *Garcinia Tetranda* Pierre Fruit Peel

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Abstract: The large genus Garcinia has encouraging bioactivity. However, the traits of many Garcinia species have not been studied thoroughly. This study was to analyze the antioxidant and anti-inflammatory activities of ethyl acetate extract of Wadung fruit skin in vitro. Antioxidant activity was determined using the ABTS method, while in vitro anti-inflammatory tests included inhibition of heat-induced hemolysis, hypotonic-induced hemolysis, protein denaturation, antiproteinase, and cyclooxygenase. The ethyl acetate extract of Wadung fruit skin showed very strong antioxidant activity as indicated by the IC5 value of 5,005, this was supported by the high content of

phenolic and flavonoid compounds as indicated by the TPC and TFC values of 104.09 ± 1.66 mg GAE / g and 102.89 ± 1.59 mg QE / g, respectively. The results of the in vitro anti-inflammatory test also showed the potential to inhibit inflammation, as shown by the IC50 in the heat-induced hemolysis test of 1.65, hypotonic-induced hemolysis test of 3.53, protein denaturation test of 2.95, antiproteinase test of 4.61, and cyclooxygenase inhibition test of 2.02. Therefore, it can be concluded that the ethyl acetate extract of Wadung fruit skin has potential as a candidate for anti-inflammatory drug.

Keywords: Hemolysis, denaturation, Garcinia tetranda, antiproteinase

乙酸乙酯提取物藤黄果皮的体外抗氧化和抗炎特性

摘要：藤黄属植物具有令人鼓舞的生物活性。然而，许多藤黄属植物的特性尚未得到彻底研究。本研究旨在分析瓦东果皮乙酸乙酯提取物的体外抗氧化和抗炎活性。抗氧化活性采用ABTS方法测定，而体外抗炎试验包括抑制热诱导溶血、低渗诱导溶血、蛋白质变性、抗蛋白酶和环氧合酶。瓦东果皮乙酸乙酯提取物表现出非常强的抗氧化活性，IC5值为5,005，这得益于高含量的酚类和黄酮类化合物，TPC和TFC值分别为104.09 ± 1.66 mg GAE / g和102.89 ± 1.59 mg QE/g。体外抗炎试验结果也显示出抑制炎症的潜力，热诱导溶血试验IC50为1.65，低渗诱导溶血试验IC50为3.53，蛋白质变性试验IC50为2.95，抗蛋白酶试验IC50为4.61，环氧合酶抑制试验IC50为2.02。因此，可以得出结论，瓦东果皮乙酸乙酯提取物具有作为抗炎药物候选物的潜力。

关键词：溶血、变性、四肽藤黄、抗蛋白酶

1. Introduction

Inflammation is a protective physiological response to infection as a body protection mechanism to neutralize and encircle harmful agents in the body by attracting plasma proteins and phagocytes to the inflamed body part, cleaning up the remains and preparing the tissue for the healing process [1]. Toxic substances that can cause inflammation include alcohol, cigarette smoke, heavy metals, air pollutants, and pesticides [2]. Inflammation is the natural response of the body to allergies. Exposure to heavy metals can affect the innate and adaptive immune systems by triggering inflammatory responses. Inflammation reduces or localizes both damaging agents and damaged tissue [3]. Some heavy metals tend to induce autoimmunity and inflammation in a similar manner. Recent research has explained the exposure of heavy metals to inflammatory reactions, namely the heavy metal cadmium (Cd), illegal drugs such as narcotics and alcohol [4].

Inflammation can be divided into two categories: acute and chronic. During the inflammatory process, several chemical mediators are released through the arachidonic pathway, namely prostaglandins, as a result of the breakdown of arachidonic acid by the cyclooxygenase enzyme [5]. Cell damage occurs when prostaglandins are released. The first stage of the arachidonic pathway begins with the release of arachidonic acid from the phospholipid membrane by phospholipase A2. In the cyclooxygenase (COX) pathway, COX converts arachidonic acid into prostaglandins [6]. Erythrocyte cells are widely used as a model to study the interactions between drugs and membranes. Erythrocytes or red blood cells have a shape similar to a biconcave disc without a nucleus and contain hemoglobin (Hb), which functions to transport oxygen or as a means of transporting oxygen in the body. The stabilization of erythrocyte cell membranes by a compound that can prevent hemolysis can be used as a benchmark for compounds that have anti-inflammatory effects [7]. Protein denaturation causes inflammation. Protein denaturation occurs because of the presence of electrostatic, hydrogen, hydrophobic, and disulfide bonds [8].

Inflammation can affect the expression and activity of proteinases in the body. During inflammation, proteinases are released by inflammatory cells such as neutrophils, macrophages, and epithelial cells. Excessive proteinase activity can cause further damage to tissues and organs. To counteract the negative effects of proteinases, the body responds by increasing their production. Antiproteinases act as regulators to prevent excessive tissue damage caused by proteinases that are left during inflammation [8].

The Guttiferae family includes the genus Garcinia, which includes approximately 450 species spread across tropical Asia, southern Africa, and western Polynesia. According to previous research, the primary compounds found in Garcinia plants are triterpenoids, flavonoids, biphenyls, xanthones, and phloroglucinols. These substances have several advantageous properties such as anti-inflammatory properties [9]. Based on this description, it is necessary to conduct an in vitro anti-inflammatory test on G. tetranda Pierre, which includes

hemolysis inhibition, protein denaturation inhibition, anti-proteinase, and cyclooxygenase inhibition tests on the ethyl acetate extract of Wadung fruit peel, because there are no data on in vitro anti-inflammatory tests. on the sample.

2. Materials and Methodes

The Wadung plant (*Garcinia tetranda* Pierre) was identified at the Botany Laboratory, Directorate of Scientific Collection Management, National Research and Innovation Agency, Bogor, Indonesia. The materials used in this study included wadung fruit peel, ethyl acetate, 0.001% trypsin, 1 ml of Tris HCL buffer 1 mM (pH 7.4), 0.02% casein (w/v), 2% pechloric acid, NSAID drugs (sodium diclofenac) methanol, dilute ammonia, Dragendroff reagent, Mayer reagent, concentrated HCl, 2N HCl, $FeCl_3$, Chloroform, concentrated sulfuric acid, gallic acid, Folin Ciocalteu, Na_2CO_3, CH_3COOH, sodium acetate, quercetin powder, $AlCl_3$, pro analysis ethanol, glacial acetic acid, phosphate buffer pH 7.0 (1 ml), hyposaline (2 ml), and (Red Blood Cell) RBC suspension (0.5 nm). This study was approved by the Health Research Ethics Committee of Universitas Nahdlatul Ulama Surabaya (number 0177/EC/KEPK/UNUSA/2024). The methodology used in this study is summarized in Figure 1.

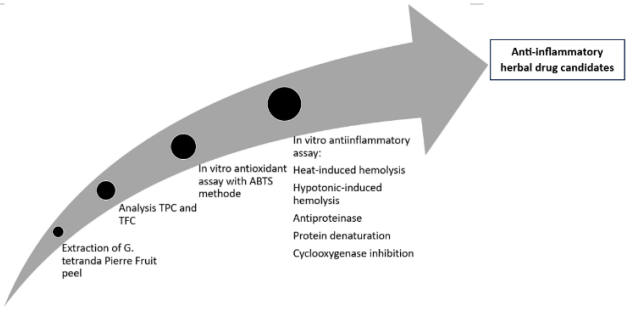


Figure 1. Research Flowchart (developed by the authors)

2.1 Extract Preparation

The peel of the Wadung fruit (*Garcinia tetranda* Pierre) was dried in an oven and then blended until it was smooth. The ethyl acetate solvent was added until it was completely submerged (1:10). Maceration was carried out for 3 × 24 h with periodic stirring. The maceration results were filtered, and the filtrate was collected using Whatman filter paper and concentrated using a rotary evaporator at a temperature of 50°C. The samples were stored in a dark glass bottle at room temperature. The thick extract was diluted to concentrations of 100, 200, 300, 400, and 500 µg/mL.

2.2 Antioxidant Assay

With a few minor adjustments, the protocol from earlier research was used to conduct the ABTS assay [10]. Equal quantities of 2.45 mM potassium persulfate and 7 mM ABTS stock solution were combined to create ABTS radical cations. Briefly, 0.360 g of ABTS salt was dissolved in 100 mL of distilled water to obtain a 7 mM ABTS solution. Salt (0.066 g) was dissolved in 100 mL of purified water to get 2.45 millimolar potassium persulfate. Next, 10 mL of a 7 mM ABTS solution and 10 mL of a 2.45 mM potassium persulfate solution were gradually mixed to create the ABTS radical cation solution. The mixture was then allowed to sit at room temperature in the dark for 12 h or until the reaction was complete, and the absorbance was stable. A UV-Vis Spectrophotometer was used to modify the absorbance value to 0.700 at 734 nm by diluting the radical cation generated in a 1:1 ratio with ethanol. Approximately 5 µL of V. amygdalina leaf extract at concentrations of 50, 100, 150, 200, 250, and 300 µg/mL was combined with 4000 µL of ABTS+• solution and allowed to sit at room temperature for two hours in the dark. The absorbance was measured using a UV–Vis spectrophotometer at 734 nm. Methanol, ethanol, water extracts, methanol, and water were used as blank solutions. As a control, 10 mL of a combination of (20 mL methanol for the methanol extract, 20 mL ethanol for the ethanol extract, and 20 mL water for the water extract), and (7 mM ABTS, 2.45 mM K2S2O8) was utilized. Ascorbic acid was used to compare the reactivities of each solvent extract at different concentrations. A minimum of three measurements were performed for each sample. Using the following equation, the percentage of ABTS+ radical scavenging was determined for different concentrations (50–300 µg/mL) of the extract and standard: sample is the absorbance of the mixture of sample/standard extract and ABTS, and Acontrol is the absorbance of a 10 mL mixture (7 mM ABTS, 2.45 mM $K_2S_2O_8$) with blank solvent.

$$\text{ABTS \% Scavenging} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

The IC_{50} value was used to express the antioxidant activity of V. amygdalina leaf extract against ABTS+•.

2.3 Preparation of Red Blood Cell (RBC)

The blood sample obtained was separated from the plasma, washed with 0.9% saline three times, and the erythrocytes were pipetted into a beaker (10 mL). Next, 90 ml of 0.9% saline was added, and the mixture was homogenized.

2.4 Analysis of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

TPC content was measured by dissolving 10 mg of the sample in 10 mL of distilled water. Pipette 20ul of sample. Add 100uL of Folin Ciocalteu reagent, vortex the sample was vortexed for 3 min. Add 80uL mL of 7.5% Na_2CO_3, and the mixture was incubated for 30 min at room temperature. The absorbance of the samples was measured at 402 nm. To calculate the TPC, the following formula was used [11]:

$$TPC = \frac{C.V.fp}{g}$$

Information:
TPC: Total Phenolic Content
C: Phenolic Concentration (x value)
V: Volume of extract used
fp: dilution factor
g: Weight of sample used

The measurement of TFC levels begins by making a blank ethanol solution p.a 0.5 mL then adding 0.10 mL of 10% $AlCl_3$, 0.10 mL of 1M sodium acetate, 2.80 mL of distilled water. Sample testing by dissolving 0.2 grams of sample with ethanol p. a. in a 10 mL measuring flask. The sample solution (0.5 mL) was then pipetted into a test tube. Add 0.10 mL 10% $AlCl_3$, 0.10 mL of 1 M sodium acetate and 2.80 mL and distilled water. The absorbance of the sample was measured at a wavelength of 433 nm. The following formula was used to calculate the TFC [11]:

$$TFC = \frac{C.V.fp}{g}$$

Description:
TPC: Total Flavonoid Content
C: Phenolic Concentration (x value)
V: Volume of extract used
fp: dilution factor
g: Weight of sample used

2.5 Heat-Induced Hemolysis Inhibition Test

The heat-induced erythrocyte hemolysis inhibition test was performed according to the study conducted by Aidoo et al., 2021, with several modifications [12]. A 1 ml pipette containing 10% erythrocyte suspension was inserted into a test tube, and 1 ml of 0.9% saline was added and then homogenized as a negative control. A 1 ml pipette containing 10% erythrocyte suspension was inserted into a test tube and 1 ml of NSAID (sodium diclofenac) 100 µg/ml was added and homogenized. sodium diclofenac 200-500 µg/mL as a positive control. One milliliter of 10% erythrocyte suspension was pipetted into a test tube and 1 ml of extract concentrations of 100, 200, 300, 400, and 500 µg/ml was added to each tube and then homogenized as a test sample. All prepared tubes were incubated for 30 min at 56°C. The solution was cooled with running water and centrifuge at 2500 rpm for 5 min. The supernatant was placed in a cuvette and the absorbance was read using a UV-Vis spectrophotometer (Thermo Scientific Genesys 10S) at a wavelength of 560 nm. Percentage inhibition was calculated using the following formula:

$$\% \text{ Inflammation Inhibition} = \frac{Abs._{control} - Abs._{sample}}{Abs_{control}} \times 100$$

The IC_{50} value was obtained from the concentration at 50% inhibition percentage, which was obtained using a linear equation.

2.6 Hypotonicity-Induced Hemolysis Inhibition Test

The hypotonicity-induced hemolysis inhibition test refers to the study [12] with several modifications. One milliliter of phosphate buffer, 2 ml of hyposaline solution and 0.5 ml erythrocyte suspension were used as negative controls. Positive control using Sodium Diclofenac 100-500 µg/mL plus 1 ml of phosphate buffer, 2 ml of hyposaline solution and 0.5 ml erythrocyte suspension. Pipette extracts with concentrations of 100, 200, 300, 400 and 500 µg/mL into each tube and add 1 ml of phosphate buffer, 2 ml of hyposaline, and 0.5 ml of erythrocyte suspension. All tubes were incubated at 37 °C for 30 min and centrifuged at 3000 rpm. The supernatant was placed in a cuvette and the absorbance was read using a UV-Vis spectrophotometer (Thermo Scientific Genesys 10S) at a wavelength of 560 nm. The percentage inhibition was calculated using the formula used in the heat-induced hemolysis inhibition test. Based on the regression curves, the concentration of the inhibitor that inhibited hypotonic-induced hemolysis by 50% (IC_{50}) was determined.

2.7 Antiproteinase Test

The antiproteinase test was performed according to the method described by Rastogi et al. (2018). Make a reaction mixture (2 ml) containing 0.001% trypsin as much as 0.06 mg, 1 ml of 1 mm Tris HCl buffer (pH 7.4), and 1 ml of test sample with concentrations of 100, 200, 300, 400 and 500 µg/ml. A standard solution containing 0.06 mg trypsin, 1 ml of 1 mm Tris HCl buffer (pH 7.4), and 1 ml of the Na-diclofenac standard. A blank solution containing 1 ml of 1 mM Tris HCl buffer (pH 7.4) was then added. The same treatment was then added to the reaction mixture with a sample concentration of 100-500 ppm and the standard with a concentration of 100-500 ppm, namely, the mixture was incubated at 37°C for 5 min, and 1 ml of 0.02% (w/v) casein was added. The mixture was then incubated for 20 min at 37°C. Subsequently, 1 ml of 2% pechloric acid was added to stop the reaction. The cloudy suspension was centrifuged, the absorbance of the supernatant was read at 210 nm against the buffer as a blank, and the percentage of proteinase activity inhibition was calculated using the formula above [13].

2.8 Protein Denaturation Inhibition Test

The protein denaturation inhibition test was carried out as described in [14] with some modifications. This test begins by making a 0.2% Bovine Serum Albumin (BSA) solution by putting 0.2 grams of BSA into a 100 mL measuring flask, then dissolving it to the boundary mark with TBS (Tris Buffer Saline) solution (1.51 grams of 0.05 M Tris and 2.1 grams of 0.15 M NaCl dissolved in 250 mL of distilled water). Subsequently, homogenize and adjust the pH of the solution to 6.6 with HCl solution (0.2% BSA buffer solution). One milliliter of 0.2% BSA was added to each test tube, 1

mL of methanol (positive control) was added to the test tube, 1 mL of standard sodium diclofenac (C14H10CI2NNaO2) 100, 200, 300, 400, and 500 µg/mL for the standard group, and 1 mL of Wadung fruit peel extract sample (100, 200, 300, 400, and 500 µg/mL). The mixture was then incubated for 20 min at room temperature, heated in a water bath at 72°C for 5 min, and cooled at room temperature. The level of protein precipitation was measured at 660 nm using a UV-Vis spectrophotometer (Thermo Scientific Genesys 10S) with a methanol blank. The percentage inhibition of protein denaturation was measured using the formula described above. IC50 values were calculated using the same method used for hemolysis inhibition [14].

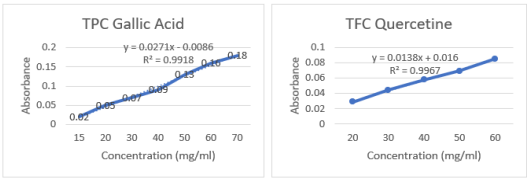
2.9 Cyclooxygenase Enzyme Inhibition Test

The cyclooxygenase enzyme inhibition test was carried out according to the procedure described by Alam et al., with several modifications. It began with the preparation of arachidonic acid, which was used as the substrate. The standard COX solution contained 50 µL of ovine COX-1 and was stored on ice (4°C) for 5-6 minutes beforehand. The cofactor solution contained 300 µL of hemin in dimethyl sulfoxide (DMSO) and the colorimetric substrate solution contained tetramethyl-p-phenylenediamine dihydrochloride (TMPD). Then, 60 µL of the standard enzyme solution was taken and reacted with 50 µL of the sample solution with concentration variations of 100, 200, 300, 400, and 500 µg/mL in each tube. The reaction was initiated when 20 µL of 30 mM arachidonic acid was added. The mixture was incubated at 37°C for 15 min. Optical density (OD) was measured using a Microplate ELISA Reader (Rayto RT-2100C) at 450 nm after the process was stopped by adding stop solution. Diclofenac sodium was used as the standard control. The percentage inhibition of cyclooxygenase enzyme was measured using the formula above. IC50 values were calculated using the same method used for hemolysis inhibition [15].

3. Results

3.1 TPC and TFC Test Results

TPC testing was performed using Folin-Ciocalteu reagent at a wavelength of 402 nm, and the results were expressed as 1 mg of gallic acid equivalent per 1 g of extract (mg GAE/g extract) using a linear regression equation. The calibration curve obtained using the gallic acid standard (R2 = 0.9918) is shown in Figure 2(a). The total phenolic content in the ethyl acetate extract of Wadung fruit peel (Garcinia tetranda Pierre) was 104.09 ± 1.66 mg GAE/g. TFC testing was carried out using aluminum trichloride ($AlCl_3$) reagent at a wavelength of 433 nm, and the results were expressed in milligrams of quercetin equivalent per 1 g of extract (mg QE/g extract). The calibration curve was obtained using the quercetin standard (R2 = 0.9967), as shown in Figure 2(b). The total flavonoid content in the ethyl acetate extract of Wadung fruit peel (Garcinia tetranda Pierre) was 102.89 ± 1.59 mg QE/g.



(a) (b)

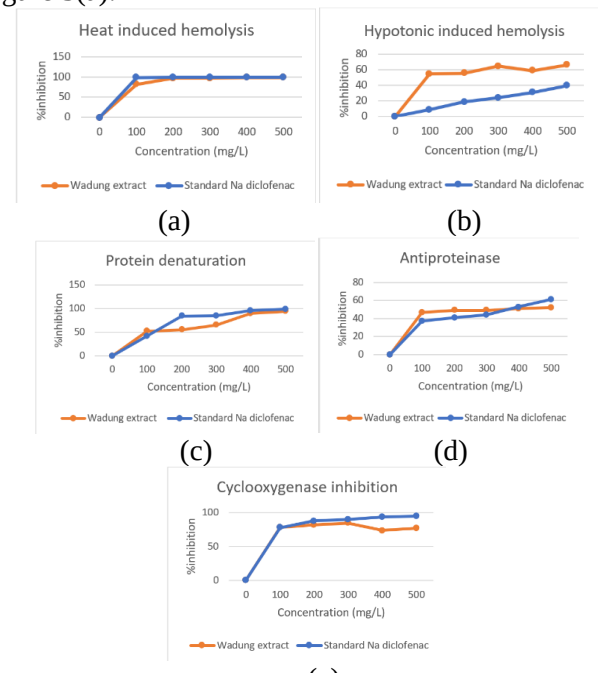
Figure 2. Callibration curve TPC and TFC (a) Total Phenolic Content (TPC); (b) Total Flavonoid Content (TFC). Source: Primary Data, 2024

3.2 Antioxidant Activity Test Results

The antioxidant activity test was carried out using the ABTS (2,2-Azinobis(3-ethylbenzothiazoline)-6-sulfonic acid) method, which refers to the research of Wairata, 2022. ABTS stabilizes free radicals through proton donors and provides lipophilic and hydrophilic compounds that can be used to evaluate the free radical-scavenging activity of extracts, using ascorbic acid as a standard. The results in Table 1 show that the IC_{50} value of the ABTS antioxidant test on the ethyl acetate extract was 5.005, whereas the IC_{50} value for the ascorbic acid standard was 2.647, indicating a very strong antioxidant potential.

3.3 Heat-induced Hemolysis Inhibition Test Results

The results of the analysis of hypotonic-induced hemolysis inhibition by the ethyl acetate extract of Wadung fruit peel are presented based on the inhibition percentage in Table 1. A comparison graph between the sample and sodium diclofenac standard is shown in Figure 3(a).



(a) (b) (c) (d) (e)

Figure 3. Comparison chart between Wadung fruit

skin ethyl acetate extract samples and Na diclofenac standard in each test (a) inhibition of heat-induced hemolysis; (b) inhibition of hypotonic-induced hemolysis; (c) inhibition of protein denaturation; (d) inhibition of antiproteinase; (e) inhibition of cyclooxygenase (COX 1/2) Source: Primary Data, 2024

It can be seen that the ethyl acetate extract of Wadung fruit peel significantly inhibits erythrocytes induced by heat. The most significant inhibition percentage shows a figure of 98.6 ± 0.000% at a concentration of 500 µg/mL. The IC_{50} value showed that there was strong inhibition in the Wadung fruit peel ethyl acetate extract sample and the standard drug Na diclofenac, namely 1.65 and 1.21, respectively.

3.4 Hypotonic Induced Hemolysis Inhibition Test Results

The effect of hypotonic-induced hemolysis inhibition by the ethyl acetate extract of Wadung fruit peel is presented based on the inhibition percentage in Table 1. A comparison graph between the sample and sodium diclofenac standard is shown in figure 3(b). These results demonstrated that the ethyl acetate extract of Wadung fruit peel inhibited hypotonicity-induced red blood cell production. The most significant inhibition percentage shows a figure of 66.06 ± 0.019% at a concentration of 500 µg/mL. As shown in Table 1, the IC_{50} values of the ethyl acetate extract of Wadung fruit peel and the standard drug Na diclofenac were 3.53 and 7.38, respectively. Although the IC_{50} value of the sample was smaller than that of the standard, both were included in the very strong inhibition category, with an IC_{50} value <50.

Table 1. Result of in vitro anti-inflammatory assay of Wadung extract and standard drugs (Source: Primary Data, 2024)

	Concentration (µg/mL)	Inhibition of Heat-Induced Hemolysis (%)	Inhibition of Hypotonic-Induced Hemolysis (%)	Inhibition of Protein Denaturation (%)	Antiproteinase	Inhibition of Cyclooxygenase (%)
Wadung Extract	100	81.1 ± 0.002	54.23 ± 0.062	52 ± 2.082	47 ± 1.332	78.14 ± 0.40
	200	97 ± 0.001	55.13 ± 0.062	55 ± 1.732	49 ± 0.851	81.55 ± 3.17
	300	97.4 ± 0.001	64.13 ± 0.026	65 ± 1.732	49 ± 0.152	84.51 ± 4.06
	400	98.5 ± 0.001	58.53 ± 0.072	90 ± 0.000	51 ± 0.435	73.76 ± 15.25
	500	98.6 ± 0.000	66.06 ± 0.019	94 ± 0.577	52 ± 0.586	76.78 ± 4.29
IC_{50}		1.65	3.53	2.95	4.61	2.02
Standard drugs Na-diclofenac	100	98.7 ± 0.000	8.12 ± 0.002	42 ± 2.082	37 ± 5.254	77.67 ± 3.48
	200	98.8 ± 0.000	18.31 ± 0.089	84 ± 0.577	41 ± 0.100	87.73 ± 11.47
	300	98.9 ± 0.001	23.9 ± 0.000	85 ± 0.577	44 ± 4.142	89.81 ± 5.22
	400	99.1 ± 0.002	30.6 ± 0.014	96 ± 0.577	53 ± 5.918	93.53 ± 5.12
	500	99.2 ± 0.001	39.33 ± 0.001	99 ± 0.000	61 ± 0.264	94.42 ± 4.15
IC_{50}		1.21	7.38	2.56	4.58	1.89

3.5 Protein Denaturation Test Results

Anti-inflammatory activity was tested in vitro using the BSA denaturation method with heat. The protective effect of the ethyl acetate extract of Wadung fruit peel is reported as the percentage of inhibition, as shown in Table 1, and a comparison graph with the standard drug, N diclofenac, is presented in figure 3(c). Based on the results obtained, it is known that the highest % inhibition of protein denaturation in the ethyl acetate extract of Wadung fruit peel was 94 ± 0.577% at a concentration of 500 µg/mL, with results that are close to or almost the same as that of the standard drug Na diclofenac, which is 99 ± 0.000%. The IC_{50} values for protein denaturation inhibition in samples and standards are known to have almost the same value, namely 2.95 and 2.56, respectively, which indicates strong inhibition.

3.6 Antiproteinase

The antiproteinase activity of the ethyl acetate extract of Wadung fruit peel ranged from 47% to-52% as shown in Table 1). A comparison graph of antiproteinase activity between the ethyl acetate extract samples of Wadung fruit peel and the standard drug, Na diclofenac, is shown in figure 3(d). The highest percentage of antiproteinase inhibition was 52 ± 0.586% at a concentration of 500 µg/mL, but the difference was 9 points lower than the concentration of 500 µg/mL for the standard drug Na diclofenac, which had a percentage of inhibition of 61 ± 0.264%. The IC_{50} values for antiproteinase activity in the Wadung fruit peel ethyl acetate extract sample and the standard drug, Na diclofenac, were 4.61 and 4.58, respectively, with values that were not significantly different, and both had very strong antiproteinase activity.

3.7 Cyclooxygenase inhibition test results

Based on the results in Table 1, the percentage of COX-1 and COX-2 inhibition was determined. The results shown in figure 3(e) show that the highest percentage of inhibition in the Wadung fruit rind ethyl acetate extract sample was 84.51 ± 4.06% at a concentration of 300 µg/mL, whereas in the Na diclofenac standard, the highest percentage of inhibition was 94.42 ± 4.15% at a concentration of 500 µg/mL. The IC_{50} values in the Wadung fruit rind ethyl acetate extract and the Na diclofenac standard were 2.02 and 1.89, respectively, indicating that both compounds exhibited strong inhibition.

4. Discussion

Based on previous research conducted by Rifaldi, 2023, it was found that the TPC test results for Garcinia bancana methanol extract samples were 195.75 ± 1.24, whereas the TFC test results were 82.79 ± 0.34 mg QE/g. This indicates that the total phenolic content is greater than the total flavonoid content because flavonoids are part of the phenolic compound [16]. Variations in TPC and TFC results can be caused by climatic conditions, geographic location, soil fertility, cultivar genotype, and research factors, such as the part of the plant used, harvest time, extraction method, solvent polarity, and extraction duration [17].

The results showed that the IC_{50} value of the ABTS antioxidant test on the ethyl acetate extract was 5.005, whereas the IC_{50} value for the ascorbic acid standard was 2.647, indicating a very strong antioxidant potential. This strong antioxidant activity is supported by optimal conditions such as growth factors, metabolism, and plant maintenance mechanisms. These conditions create an environment in which plants can produce and accumulate high levels of antioxidant compounds in the extract. In addition, the production of active antioxidant compounds is influenced by nutrient availability. Under optimal conditions, plants have access to essential nutrients such as minerals and vitamins, which play important roles in antioxidant synthesis. Under favorable environmental conditions, plants can increase the expression of genes involved in the synthesis of certain antioxidant compounds [18].

Previous studies have shown that the extract has anti-inflammatory activity when it stabilizes the membrane. Hemolysis is inhibited through the inhibition of the release of lysosomal contents from neutrophils at the site of inflammation [19]. Nwankwo et al. [20] also explained that the lysosomal membrane is susceptible to damage by agents that modulate the inflammatory response. Excessive proteolytic enzymes can be released during lysosomal injury, triggering inflammation. Therefore, if the extract can stabilize the red blood cell membrane, it has the potential to stabilize the lysosomal membrane [20]. Based on the research results, it is known that the percentage of inhibition of heat-induced hemolysis in the ethyl acetate extract of Wadung fruit peel is not much different from that of the standard drug Na diclofenac; therefore, it can be said that the sample has the potential to inhibit the inflammatory process through inhibition of hemolysis.

Protection of an active substance against heat-induced protein denaturation caused by heat can be considered a mechanism of anti-inflammatory activity. Protein denaturation is caused by changes in hydrogen, hydrophobic, electrostatic, and disulfide bonds, resulting in the loss of secondary and tertiary structures and triggering inflammation [21]. The maintenance of the structural and functional integrity of cells and their internal constituents is strongly dependent on proteins. Inflammation is caused by the structural alterations that occur when proteins are denatured. Enzymes such as phospholipase A2, which break down phospholipids in cell membranes, are released during inflammation. This leads to the discharge of cellular content, which causes inflammation and tissue damage. An important measure of anti-inflammatory potential is inhibition of protein denaturation. The ability of the material to preserve and maintain cell membranes was evaluated using a membrane stabilization assay. The protein albumin denaturation assay assesses the ability of a compound to prevent protein denaturation. Consequently, substances or medications that can prevent protein denaturation are considered potentially valuable in terms of their anti-inflammatory properties [22]. This study found that the percentage of inhibition of protein denaturation from the ethyl acetate extract of Wadung fruit peel was not significantly different from that of Na diclofenac; therefore, it can be said that the sample has the potential to be anti-inflammatory.

Because leukocyte proteinases are crucial in the development of inflammatory processes that cause tissue damage, proteinase inhibitors, such as bioactive substances, are expected to provide a high degree of protection. Consequently, plants containing bioactive compounds may exhibit anti-inflammatory properties [23]. Many serine proteinases are carried by leukocytes in their lysosomal granules. For instance, during inflammation, leukocytes release lysosomal enzymes, including proteinases, as part of their protective function, resulting in increased tissue damage and recurrent inflammation. Atherosclerosis can result from secondary damage caused by free radical-induced lipid peroxidation, which is more likely to occur in cells with damaged membranes [24]. From this study's ethyl acetate extract of Wadung fruit peel, it can be inferred that the sample can block the release of leukocyte lysosomal components at the site of inflammation, because it exhibited antiproteinase activity comparable to that of sodium diclofenac.

COX-2 is the primary enzyme that is involved in inflammation. This inducible enzyme then produces inflammatory mediators. In most tissues, COX-2 is

virtually nonexistent under normal circumstances; nevertheless, its expression can increase dramatically from 10 to 80 times when inflammation arises or following exposure to mutagenic stimuli. By blocking constitutive COX-1 and inducible COX-2, most commercially available nonsteroidal anti-inflammatory drugs (NSAIDs) decrease the formation of prostaglandins (PGs) and thromboxane (TX). The suppression of COX-2 is most likely the cause of the anti-inflammatory, analgesic, and antipyretic effects of NSAIDs [25]. Arachidonic acid (AA) is the most important precursor of the inflammatory pathway. Internal and external factors trigger the activation of phospholipase-A2. Membrane-bound AA is broken down by this activation, which opens it up for COX. Pro-inflammatory enzymes, such as COX and LOX, are well known and necessary for the physiological synthesis of eicosanoids, prostaglandins, and leukotrienes. COX oxidizes arachidonate to produce PGG2, which can be oxidized to produce a PGH2 precursor and other PGs such as thromboxane, which are crucial to the process of inflammation [26]. Based on the data in Table 1, the ethyl acetate extract of the wading fruit peel has good cyclooxygenase inhibitory and anti-inflammatory activities.

5. Conclusion

Based on an IC_{50} value of 5,005, the Wadung fruit skin ethyl acetate extract demonstrated strong antioxidant activity. This was further supported by the high content of flavonoid and phenolic compounds, as shown by the TPC and TFC values of 102.89 ± 1.59 mg QE/g and 104.09 ± 1.66 mg GAE/g, respectively. The IC_{50} values obtained from the heat-induced hemolysis test was 1.65, the hypotonic-induced hemolysis test was 3.53, the protein denaturation test was 2.95, the antiproteinase test was 4.61, and the cyclooxygenase inhibition test 2.02 all indicated that the in vitro anti-inflammatory test results have the potential to inhibit inflammation.

Previous studies have identified the antioxidant and antibacterial activity of Garcinia *tetranda* Pierre stem barks, but no research has been found on the anti-inflammatory activity of the fruit skin extract. Therefore, this research will have an impact on the exploration of bioactivity of Garcinia species. This study has found the potential for in vitro antioxidant and anti-inflammatory activity of *G. tetranda* Pierre extract, but it still needs to be continued for in vivo testing. Therefore, further research is recommended to conduct in vivo anti-inflammatory activity testing.

Declarations

Author Contributions

Conceptualization, D.D.W.; methodology, S.N.C.; validation, N.R.; formal analysis, I.E.; investigation, H.; resources, S.R.; data curation, N.C.; writing—original draft preparation, all authors contributed equally; writing—review and editing, M.S.; visualization, A.F.; supervision, D.D.W.; project administration, A.P.R.S. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

The data presented in this study are available in this article.

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Institutional Review Board Statement

The study was approved by the Health Research Ethics Committee of Universitas Nahdlatul Ulama Surabaya (Number 0177/EC/KEPK/UNUSA/2024).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

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