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In vitro Evaluation of Anti-inflammatory Protein-Denaturation Activity of Cashew (*Anacardium occidentale* Linn) Stem Bark Using a UV–Vis Spectrophotometer

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Abstract: Inflammation is a protective response of the body caused by tissue damage due to abnormal physical conditions, harmful chemical compounds, or microbiological agents. The protein denaturation method for anti-inflammatory testing aims to determine whether the ethyl acetate and ethanol–water fractions of cashew (*Anacardium occidentale* Linn) stem bark exhibit anti-inflammatory activity using the protein denaturation method, and to determine the IC_{50} values of both fractions. The anti-inflammatory activities of the ethyl acetate and ethanol–water fractions, along with the positive control, were tested using a UV–Vis spectrophotometer at concentrations of 100 ppm, 200 ppm, 400 ppm, 800 ppm, and 1600 ppm. The results showed that the ethyl acetate fraction of cashew stem bark (*Anacardium occidentale* L.) had an IC_{50} value of 531.74 µg/mL, the ethanol–water fraction had an IC_{50} value of 101.31 µg/mL, and the positive control had an IC_{50} value of –70.85 µg/mL. These findings indicate that the ethanol–water fraction exhibited moderate anti-inflammatory activity, while the ethyl acetate fraction showed weak activity.

Keywords: *Anacardium occidentale*; Anti-inflammatory; In-vitro; UV–Vis Spectrophotometry.

Introduction

Inflammation is a protective response of the body that arises due to tissue damage caused by abnormal physical conditions, microbiological agents, or harmful chemical substances. The primary functions of inflammation are to destroy, reduce, or limit the effects of the damaging agent or the injured tissue. Swelling, redness, heat, pain, and alteration in tissue function are the classical signs of inflammation (Ramadhani & Sumiwi, 2016). Along with the advancement of research on plant-derived anti-inflammatory agents, there has been a growing public preference and trust toward traditional medicine, as it is believed to be safer and to cause fewer side effects compared to synthetic drugs.

Several plants are empirically believed by the community to possess anti-inflammatory properties, one of which is the stem bark of the cashew tree (*Anacardium occidentale* Linn). Cashew, scientifically known as *Anacardium occidentale* Linn, is a medicinal plant with various benefits derived from almost all parts, including the leaves, roots, and fruits. Research on the phytochemical content of cashew stem bark using ethanol extraction has shown that it contains anacardia acid, gallic acid, flavonoids, and saponins (Harsini, 2017). In a study by Veriony et al., (2011), the relative concentration of anacardia acid was found to be 0.21%.

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After spiking the decoction with an anacardia acid standard, the relative concentration increased from 0.21% to 0.36%. These findings confirm the presence of anacardia acid compounds in the cashew stem bark decoction. Based on the phenolic content of cashew stem bark, Choi et al. (2019), reported that anacardia acid exhibits antimicrobial, anti-inflammatory, antimalarial, and antioxidant activities, as well as inhibitory effects on several enzymes such as xanthine oxidase, lipoxygenase, and cyclooxygenase, which are commonly found in the bark of plants belonging to the *Anacardium* genus.

In the study conducted by (Nirmala Aga, 2018), the total flavonoid content of the ethanol extract from cashew (*Anacardium occidentale* Linn) stem bark was reported to be 89,358 mg QE/100 g ± 7.704%. According to Nirmala, the ethanol extract was obtained through maceration using 70% ethanol as the solvent, yielding a rendement of 22.02% w/w. The total flavonoid content of the ethanol extract was found to be 89,358 mg QE/100 g ± 7.70. Various studies have reported that cashew contains several bioactive compounds, one of which is flavonoids, known for their anti-inflammatory properties. According to flavonoids act as anti-inflammatory agents by inhibiting the cyclooxygenase and lipoxygenase enzymes, thereby providing potential therapeutic benefits for the treatment of inflammation and allergic reactions.

Several research findings have reported similar results (Prayitno et al., 2022), demonstrated that the stem bark extract of cashew (*Anacardium occidentale* Linn) exhibits anti-inflammatory effects in mice. Among the tested concentrations of 2% b/v, 4% b/v, and 8% b/v, the 8% b/v concentration produced the most significant anti-inflammatory effect, with a percentage reduction of 78.86% in *Mus musculus*. In another study, Veriony et al., (2011), reported that the decoction of cashew stem bark exhibited anti-inflammatory activity in rats at concentrations of 12% b/v (1.25 g/kg BW), 25% b/v (2.5 g/kg BW), and 50% b/v (5 g/kg BW), yielding respective inhibition values of 40.22 ± 11.70%, 46.80 ± 9.46%, and 44.33 ± 6.24%. These anti-inflammatory activities did not show a significant difference compared to indomethacin at a dose of 10 mg/kg BW, which exhibited an inhibition of 56.85 ± 6.34%. That the ethyl acetate extract of cashew pseudofruit (*Anacardium occidentale* Linn) at doses of 450 mg/kg BW, 900 mg/kg BW, and 1.8 g/kg BW showed anti-inflammatory effects on paw edema in male Wistar rats induced with 0.5% carrageenan (0.5 mL). The combined administration of the ethyl acetate extract (900 mg/kg BW) with sodium diclofenac (3.357 mg/kg BW) resulted in a lower anti-inflammatory effect compared to the extract alone at doses of 450 mg/kg BW and 900 mg/kg BW. The reduction in edema volume induced by the pseudofruit extract at doses of 450 mg/kg BW, 900 mg/kg BW, and 1.8 g/kg BW was lower than that of sodium diclofenac. These findings indicate that *Anacardium occidentale* Linn extract possesses anti-inflammatory activity.

Several methods can be used to isolate and separate one or more chemical components, including extraction and fractionation. Extraction is a separation technique used to isolate or withdraw one or more analytes from a sample using an appropriate solvent (Rahmi et al., 2020). Fractionation, on the other hand, is a process of separating compounds from an extract by using two immiscible solvents. The solvents commonly used for fractionation include *n*-hexane, ethyl acetate, and methanol. *n*-Hexane is typically used to extract fats and non-polar compounds, ethyl acetate for semi-polar compounds, and methanol for polar compounds. Through this process, the polarity characteristics of the separated compounds can be predicted. It is well known that non-polar compounds dissolve in non-polar solvents, while polar compounds dissolve in polar solvents (Ellita et al., 2025).

This study on anti-inflammatory activity was conducted using an in vitro method. The in vitro approach offers several advantages, including shorter testing time, the use of smaller sample quantities, and the absence of animal testing requirements (Jain et al., 2018). One of the in vitro methods that can be employed is the protein denaturation method (Samarakoon, 2023). Protein denaturation in tissues is one of the causes of inflammation. Protein denaturation refers to the process in which structural changes or modifications occur in protein molecules. The mechanism of protein denaturation involves the alteration of electrostatic, hydrogen, hydrophobic, and disulfide bonds. The inhibitory activity against protein denaturation is expressed as a percentage of inhibition (Esho et al., 2021). Moreover, since the protein denaturation assay involves heating, it is considered highly suitable for use because it is non-acidic and cost-effective.

Previous studies have demonstrated that cashew (*Anacardium occidentale* Linn) extracts contain active compounds such as anacardia acid, flavonoids, gallic acid, and saponins, which possess anti-inflammatory properties. However, most of these studies were limited to in vivo testing or did not fully explain the biochemical mechanisms underlying inflammation inhibition. Therefore, this study is significant in providing more specific and efficient experimental evidence through a rapid, cost-effective laboratory method that does not involve animal testing. The findings of this research are expected to support the development of safe, economical, and natural-based herbal anti-inflammatory agents, while also enriching scientific knowledge on the

utilization of Indonesian traditional plants in modern pharmacology.

Method

Design, Place, and Time

This study employed an experimental laboratory design to determine the anti-inflammatory activity of cashew (*Anacardium occidentale* Linn) stem bark through the protein denaturation method using a UV–Vis spectrophotometer. The research was conducted at the Phytochemistry, Instrumentation, and Pharmaceutical Chemistry Laboratory, Megarezky University, Makassar, beginning in August 2025.

Materials and Equipment

The equipment used in this experiment included a dropper pipette, test tubes, Erlenmeyer flasks, volumetric flasks, micropipettes, measuring cylinders, a blender, a water bath, a UV–Vis spectrophotometer, and a rotary evaporator. The materials used in this study were cashew (*Anacardium occidentale* Linn) stem bark, distilled water, 96% ethanol, Tris base, bovine serum albumin (BSA), glacial acetic acid, NaCl, and sodium diclofenac.

Population and Sample

The cashew (*Anacardium occidentale* Linn) plants used in this study were collected from Maros Regency, South Sulawesi. The stem bark was thoroughly washed to remove any adhering impurities. The drying process was carried out to eliminate the moisture content present in the bark, while the grinding process aimed to increase the surface area and enhance the contact between the solvent and the sample, thereby allowing optimal extraction of the bioactive compounds contained in the sample.

Extraction and Liquid–Liquid Partition

A total of 700 grams of cashew (*Anacardium occidentale* Linn) stem bark simplicial powder was weighed and placed into a container, then extracted for 2 × 24 hours using 96% ethanol as the solvent, with occasional stirring. The resulting filtrate was collected and concentrated using a rotary evaporator until a thick extract was obtained. The crude fractionation process followed the method described by Can-ake (2004), involving partitioning with 96% ethanol–water (2:3) and ethyl acetate solvents. Ten grams of the concentrated extract were dissolved in 100 mL of the ethanol–water mixture, followed by partitioning with 100 mL of ethyl acetate. The mixture was shaken in a separating funnel, left to stand for 30–60 minutes, and the formed layers were separated, with the ethanol–water layer forming the lower phase and the ethyl acetate layer forming the upper phase.

Phytochemical Screening

Approximately 0.1 gram of the ethyl acetate, n-hexane, and ethanol fractions was each dissolved in 10 mL of hot water and heated for 5 minutes. The solution was then filtered, and the filtrate was collected. One milliliter of the filtrate was placed into a test tube, followed by the addition of 0.5 grams of magnesium powder, 1 mL of concentrated HCl, and 1 mL of alcohol, then shaken gently. The appearance of a red, yellow, or orange coloration indicated a positive result for the presence of flavonoids.

Protein Denaturation Test

Preparation of Tris Buffer Saline (TBS) Solution

A total of 1.21 grams of Tris base and 8.7 grams of sodium chloride were placed into a 1,000 mL volumetric flask and dissolved in 900 mL of distilled water. The pH was adjusted to 6.2–6.5 by adding glacial acetic acid, and the solution was then made up to the mark with distilled water to a final volume of 1,000 mL.

Preparation of 0.2% Bovine Serum Albumin (BSA) in TBS

BSA (1 gram and 0.5 gram) was dissolved in 500 mL and 250 mL volumetric flasks, respectively, using Tris buffer saline (TBS) as the solvent. Each solution was then made up to the mark to obtain 0.2% BSA in TBS

Preparation of Ethyl Acetate Fraction, Ethanol–Water Fraction, and Positive Control Solutions

Each of the ethyl acetate fraction, ethanol–water fraction, and sodium diclofenac (as a positive control) was weighed at 250 mg and dissolved in separate 50 mL volumetric flasks using distilled water to obtain a stock solution of 5,000 ppm. Serial dilutions were then prepared to obtain concentrations of 1,600 ppm, 800 ppm, 400 ppm, 200 ppm, and 100 ppm..

Preparation of Negative Control

A total of 50 µL of distilled water was mixed with 0.2% BSA in a volumetric flask and made up to a final volume of 5 mL.

Measurement of Anti-inflammatory Activity

From each test solution and the positive control (sodium diclofenac), aliquots of 1 mL (100 ppm), 2 mL (200 ppm), 4 mL (400 ppm), 8 mL (800 ppm), and 16 mL (1,600 ppm) were taken and diluted to 50 mL with distilled water. The mixtures were incubated at 25°C for 30 minutes, followed by heating at 72°C for 5 minutes. The solutions were then allowed to stand at room temperature for 25 minutes. After vigorous shaking, the

absorbance was measured using a UV–Vis spectrophotometer at a wavelength of 660 nm.

Data Analysis

The analysis of in vitro anti-inflammatory activity was expressed by determining the IC_{50} (Inhibition Concentration 50) value, which represents the concentration required to inhibit 50% of protein denaturation. The IC_{50} value was obtained by constructing a linear regression equation, y = a + bx, where y represents absorbance and x represents the concentration of cashew (*Anacardium occidentale* Linn) stem bark extract. The linear graph was generated using Microsoft Excel software.

Result and Discussion

Result

In this study, the anti-inflammatory activity of the ethanol and ethyl acetate fractions from the stem bark of cashew (*Anacardium occidentale* Linn) was tested using the protein denaturation method. The cashew stem bark was collected from the Maros region, South Sulawesi. A total of 700 grams of simplicial powder was extracted using 96% ethanol for two days, yielding a concentrated extract with the following results.

Table 1. Yield of Cashew Stem Bark Extract

Total Simplicial (g)	Solvent	Total Extract (g)	% Yield
700 gram	Etanol 96%	65,74 gram	9.391429

The obtained extract was then fractionated into ethyl acetate and ethanol–water fractions.

Table 2. Yield of Cashew Stem Bark Fractions

Fraction Type	Initial Extract (g)	Total Fraction (g)	% Yield
Ethyl acetate	10 gram	082 gram	8.2
Ethanol–water	10 gram	6.89 gram	68.9

Phytochemical Screening

Phytochemical screening was performed to detect the presence of flavonoid compounds in both the ethyl acetate and ethanol–water fractions of cashew (*Anacardium occidentale* Linn) stem bark.

Table 3. Phytochemical Screening Results Flavonoid Compounds

Sample	Reagent	Observation Result	Reference Observation	Description
Ethyl acetate fraction	Wilstätter	Orange/Yellow	Red, Yellow, or Orange	Positive
Ethanol–water fraction	Wilstätter	Red	Red, Yellow, or Orange	Positive

Based on the results in the table above, both the ethyl acetate and ethanol–water fractions of cashew stem bark (*Anacardium occidentale* Linn) were confirmed to contain flavonoid compounds. According to Wijayanti & Qomariyah, (2023), he reduction reaction with magnesium and concentrated hydrochloric acid can produce red or orange coloration in flavonols, flavones, flavanonols, and xanthones. Gelian et al. (2024), also reported that flavonoid aglycones such as isoflavones, flavanones, and flavonols tend to dissolve in semi-polar solvents such as ether, chloroform, ethyl acetate, and n-butanol, while flavonoid glycosides are more soluble in polar solvents such as water.

Table 4. Results of Anti-inflammatory Test – Negative Control Absorbance

Parameter	Replication			Mean
	1	2	3	
Negative Control	0.4595	0.4593	0.4588	0.4592

Table 5. Results of Anti-inflammatory Test – Ethyl Acetate Fraction

Concentration (ppm)	Replication			Mean	Ethyl Acetate % Inhibition	lC50 (µg/mL)
	1	2	3			
100	0.3347	0.3346	0.3344	0.334567	27.1414053	531.737288
200	0.2993	0.2992	0.299	0.299167	34.8504646	
400	0.2506	0.2506	0.2505	0.250567	45.4340883	
800	0.1658	0.1658	0.1657	0.165767	63.9009872	
1600	0.0026	0.0026	0.0026	0.0026	99.4337979	

Based on the percentage inhibition values, the ethyl acetate fraction of cashew (*Anacardium occidentale* Linn) stem bark exhibited anti-inflammatory potential with inhibition values above 20%. The IC_{50} value of 531.74 µg/mL indicates that the ethyl acetate fraction possesses weak anti-inflammatory activity.

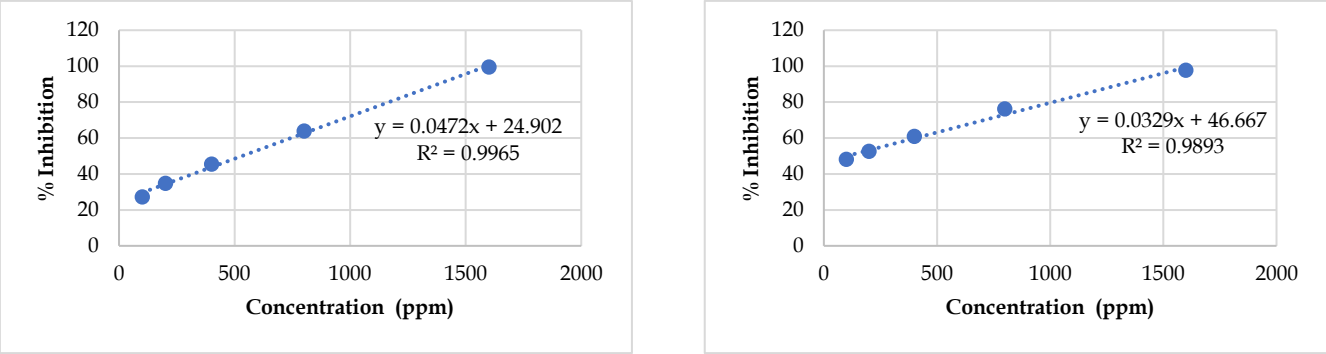

Figure 1. Graph of fraction of cashew: (a) Ethyl Acetate; and (b) Ethanol–Water

Table 6. Results of Anti-inflammatory Test – Ethanol–Water Fraction

Concentration (ppm)	Ethanol–Water					lC50 (µg/mL)
	Repetition			Mean	% Inhibition	
	1	2	3			
100	0.2383	0.2382	0.2387	0.2384	48.08362	101.306990
200	0.2176	0.2176	0.2176	0.2176	52.61324	
400	0.1799	0.1798	0.1797	0.1798	60.84495	
800	0.1097	0.1094	0.1095	0.109533	76.14692	
1600	0.0104	0.0103	0.0104	0.010367	97.74245	

Ethanol–Water Fraction

Based on the percentage inhibition values, the ethanol–water fraction of cashew stem bark showed inhibition above 20%, indicating anti-inflammatory potential. The IC_{50} value of 101.31 µg/mL suggests that the ethanol–water fraction exhibits moderate anti-inflammatory activity.

Positive Control

Based on the inhibition percentage, the positive control (sodium diclofenac) demonstrated strong anti-inflammatory potential, with inhibition values above 20%. The IC_{50} value of –70.85 µg/mL indicates very strong anti-inflammatory activity, as IC_{50} values below 50 µg/mL are classified as highly active.

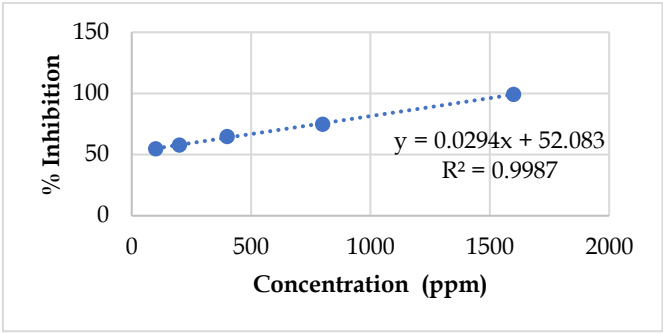

Figure 2. Sodium Diclofenac

Table 7. Results of Anti-inflammatory Test – Positive Control

Concentration (ppm)	Sodium Diclofenac					lC50 (µg/mL)
	Replication			Mean	% Inhibition	
	1	2	3			
100	0.2076	0.2077	0.2081	0.2078	54.747387	-70.85034014
200	0.1935	0.1934	0.1936	0.1935	57.861498	
400	0.1615	0.1616	0.1616	0.161567	64.815621	
800	0.1159	0.1158	0.1157	0.1158	74.78223	
1600	0.0033	0.0034	0.0035	0.0034	99.259582	

Discussion

In this study, the anti-inflammatory activity of the cashew (*Anacardium occidentale* L.) stem bark was evaluated in vitro through the protein denaturation method using a UV–Vis spectrophotometer. Inflammation is a protective response of the body triggered by abnormal physical conditions, harmful chemical compounds, or microbiological agents (Chen et al., 2018). Inflammation occurs because of the release of several inflammatory mediators through the

arachidonic acid pathway, in which prostaglandins are produced from the breakdown of arachidonic acid by the cyclooxygenase (COX) enzyme. When this process becomes excessive, inflammation can progress to an acute stage that may lead to loss of tissue function due to physical swelling, resulting in restricted tissue movement and impaired blood flow to vital organs such as the heart, which in severe cases can lead to death (Vasques-Nóvoa et al., 2022).

Inflammation triggers the release of numerous endogenous substances known as inflammatory mediators. Among these, arachidonic acid plays a crucial role in the biosynthesis of prostaglandins through the cyclooxygenase (COX) pathway. Cyclooxygenase-1 (COX-1) functions in maintaining normal physiological processes such as mucus secretion for gastrointestinal mucosal protection and the regulation of renal function. Cyclooxygenase-2 (COX-2), on the other hand, is an inducible enzyme whose expression increases in response to various stimuli, including cytokines, bacterial lipopolysaccharides, inflammation, or other pathological conditions.

Inflammation also leads to the accumulation of white blood cells, particularly neutrophils and monocytes, at the site of injury to eliminate or limit the damaging agent. Neutrophils subsequently undergo margination, emigration, chemotaxis, and phagocytosis. Controlling pain and edema enhances the immune response during wound healing, thereby promoting faster recovery. One effective way to control pain and edema is by inhibiting the cyclooxygenase enzyme (Kusumastuti et al., 2014).

The sample used in this study was cashew (Anacardium occidentale L.), selected based on previous findings by Siracusa et al., (2020), which reported that the stem bark extract of cashew exhibited anti-inflammatory effects in mice. Among the tested concentrations of 2% b/v, 4% b/v, and 8% b/v, the 8% b/v concentration demonstrated the most effective anti-inflammatory response in Mus musculus, with a reduction rate of 78.86%. The cashew stem bark used in this study was collected from Maros Regency, South Sulawesi. The stem bark was chosen as the plant part for extraction because it requires a relatively shorter extraction time compared to the leaves, which typically require up to one week of extraction. The collected stem bark samples were sorted to remove any dirt or impurities adhering to the plant material before further processing.

The drying process was carried out by air-drying the samples in the shade, avoiding direct exposure to sunlight to minimize the degradation of the plant's chemical constituents (Khoury et al., 2021). The dried bark was then ground into fine powder to reduce particle size, thereby maximizing the efficiency of the extraction process. Smaller or finer simplicial particles allow for better solvent penetration and more effective compound extraction (Baldelli & Aguilera, 2025). A total of 700 grams of the dried simplicial powder was subsequently stored in tightly sealed containers to prevent contamination by microbes and other microorganisms.

Extraction is a type of separation process that involves the transfer of one or more substances from a solid or liquid matrix. The extraction process begins with the breakdown of plant material, followed by contact between the sample and solvent, allowing diffusion to occur at the interface between the extracted material and the solvent (Hidayah & Abidin, 2017). Extraction methods are generally classified into two categories based on the use of heat: hot extraction and cold extraction (Azzahra et al., 2024).

In this study, extraction was performed using a cold extraction technique, specifically the maceration method. This method was chosen because it minimizes heat exposure, thereby reducing the risk of degradation of heat-sensitive compounds (Khoury et al., 2021). The solvent used for maceration was 96% ethanol. According to Hasyim et al. (2023), 96% ethanol is widely used as a solvent due to its universal, polar nature, ease of availability, and effectiveness in extracting a broad range of compounds. Ethanol 96% was selected because it is selective, non-toxic, has good absorbance properties, and a high extraction efficiency, allowing it to extract non-polar, semi-polar, and polar compounds. The extraction process yielded 65.74 grams of extract with a percentage yield of 9.39%.

Fractionation was carried out to separate the chemical compounds contained in the plant sample based on their polarity levels. According to Gulo et al. (2021), flavonoid aglycones such as isoflavones, flavanones, and flavanols tend to dissolve in semi-polar solvents such as ether, chloroform, ethyl acetate, and n-butanol, while flavonoid glycosides are more soluble in polar solvents such as water. The types of fractions used in this study were the ethyl acetate fraction and the ethanol–water mixture fraction in a ratio of 2:3 (2 ethanol: 3 water) (Gelian et al., 2024). A total of 10 grams of extract was fractionated using 100 mL of each solvent system, resulting in 6.89 grams of the ethanol–water fraction and 0.82 grams of the ethyl acetate fraction.

Phytochemical screening was conducted to identify the presence of flavonoid compounds in both the ethyl acetate and ethanol–water fractions. Flavonoids are known to possess anti-inflammatory or anti-phlogistic properties (Al-Khayri et al., 2022). Various studies have shown that the main compounds responsible for anti-inflammatory activity are flavonoids, which act by

inhibiting the enzymes cyclooxygenase and lipoxygenase, and by preventing leukocyte accumulation at the site of inflammation. Flavonoid compounds specifically suppress the development and recruitment of irritant-inducing mediators that trigger hypersensitivity responses. The functional groups present in flavonoids contribute to multiple mechanisms that effectively counteract inflammatory reactions.

The anti-inflammatory mitigating effects of flavonoids can occur through several pathways, one of which involves the direct inhibition of cyclooxygenase (COX) and lipoxygenase enzymes, leading to the suppression of prostaglandin and leukotriene biosynthesis — the end products of the COX and lipoxygenase pathways. This inhibition prevents leukocyte aggregation and neutrophil degranulation, thereby reducing the arachidonic acid cascade initiated by neutrophils and downregulating receptor activation. Under normal physiological conditions, leukocytes move freely along the endothelial wall; however, during inflammation, mediators released by the endothelium and other complement factors promote leukocyte adhesion to the endothelium. Flavonoid administration has been shown to reduce the number of leukocytes and limit complement formation, thus decreasing leukocyte–endothelial interactions and consequently diminishing the body's inflammatory response.

In addition, other mechanisms of flavonoid-mediated anti-inflammatory action include inhibition of arachidonic acid release, suppression of lysosomal enzyme secretion from neutrophils and endothelial cells, and inhibition of both the exudative and proliferative phases of inflammation. The inhibition of arachidonic acid release results in a reduction in the amount of substrate entering the cyclooxygenase and lipoxygenase pathways. Consequently, this leads to decreased synthesis and production of prostaglandins, prostacyclins, endoperoxides, and thromboxanes on one hand, and hydroperoxy acids and leukotrienes on the othe (Anggraeny, 2017).

The results of the phytochemical screening showed that both the ethyl acetate fraction and the ethanol–water fraction tested positive for the presence of flavonoid compounds. In the Wilstätter test, the ethanol–water fraction produced a red coloration, while the ethyl acetate fraction produced an orange–yellow coloration. According to (Zeinali et al., 2017), the appearance of red, orange, or yellow coloration indicates a positive reaction for flavonoids. Robinson also stated that the reduction reaction involving magnesium and concentrated hydrochloric acid can produce red or orange coloration in flavonols, flavones, flavanonols, and xanthones. The change from yellow to red observed in the Wilstätter test indicates the reduction of the benzopyrone ring in the flavonoid structure (Roghini & Vijayalakshmi, 2018). From these observations, it can be concluded that flavonoids are the main compounds responsible for anti-inflammatory activity. Flavonoids exert their effect by inhibiting cyclooxygenase (COX) and lipoxygenase enzymes, as well as by preventing leukocyte accumulation at the site of inflammation. These compounds specifically suppress the development and migration of irritant-inducing mediators triggered by hypersensitivity reactions. The presence of flavonoid functional groups contributes to multiple biological activities that collectively alleviate inflammation.

The anti-inflammatory mechanism of flavonoids occurs through several pathways, one of which involves the direct inhibition of COX and lipoxygenase enzymes, resulting in the suppression of prostaglandin and leukotriene biosynthesis—the final products of these enzymatic pathways. This process inhibits leukocyte aggregation and neutrophil degranulation, thereby reducing arachidonic acid metabolism and receptor activation. Under normal physiological conditions, leukocytes move freely along the endothelial walls; however, during inflammation, mediators secreted by the endothelium and complement factors promote leukocyte adhesion to endothelial cells. Administration of flavonoids reduces leukocyte count and complement formation, thereby limiting leukocyte–endothelial adhesion and diminishing the overall inflammatory response.

Anti-inflammatory studies are generally classified into two types: in vivo and in vitro assays. The term in vivo, derived from Latin meaning "within the living," refers to experiments conducted using whole living organisms rather than isolated tissues or non-living systems. Animal testing and clinical trials are examples of in vivo studies. Meanwhile, in vitro and in situ are also derived from Latin; in vitro ("in glass") refers to experimental techniques performed outside a living organism under controlled environmental conditions. This study employed the in vitro method, which offers several advantages such as shorter testing time, smaller sample requirements, and the absence of animal use. Several in vitro methods for evaluating anti-inflammatory activity include the protein denaturation assay, red blood cell (RBC) membrane stabilization assay, and platelet anti-aggregation, anticoagulant, and thrombolytic activity tests (Wirawan et al., 2021).

The in vitro assays specifically used to assess anti-inflammatory potential include the protein denaturation method (Novika, Ahsanunnisa, & Yani, 2021), and the RBC membrane stabilization method (Armadany et al., 2020). The protein denaturation method is based on the process in which proteins lose their tertiary and secondary structures upon exposure to external agents

such as strong acids or bases, organic salts, organic solvents, or heat (Mulyani et al., 2023). Protein denaturation in tissues contributes to the onset of inflammation. The RBC membrane stabilization method, on the other hand, involves assessing the stability of red blood cells when induced with hemolytic solutions. This induction causes oxidative stress, which can disrupt biomembrane stability. The extent of hemolysis observed in RBCs exposed to hypotonic solutions serves as an indicator of anti-inflammatory activity (Novika et al., 2021).

One of the methods used in this study was the protein denaturation assay. The advantage of this method is that it requires a relatively short testing time compared to the red blood cell (RBC) membrane stabilization method, which takes longer because it requires finding human donors who are not taking anti-inflammatory medications. One of the main causes of inflammation is protein denaturation within tissues. Protein denaturation refers to the interaction that leads to alterations or conformational changes in the protein structure. The process of protein denaturation involves modifications in electrostatic, hydrogen, hydrophobic, and disulfide bonds. The degree of inhibition of protein denaturation is expressed as a percentage of inhibition (Mahran et al., 2023).

The in vitro anti-inflammatory test using the protein denaturation method employed bovine serum albumin (BSA) as the negative control, since BSA is a type of protein. Sodium diclofenac was used as the positive control, as it is a standard anti-inflammatory drug commonly utilized in similar studies. The absorbance measurement using a UV–Vis spectrophotometer for the negative control (0.2% BSA) yielded an average absorbance value of 0.4592. Based on this value, the percentage of inhibition and the IC_{50} for each fraction and the positive control were calculated. The percentage inhibition values for the ethyl acetate fraction were 27.14% (100 ppm), 34.85% (200 ppm), 45.43% (400 ppm), 63.90% (800 ppm), and 99.43% (1600 ppm).

According to Hasim et al. (2019), f the percentage inhibition value exceeds 20%, the sample can be considered to possess anti-inflammatory properties. The IC_{50} value obtained for the ethyl acetate fraction was 531.74 µg/mL, indicating weak anti-inflammatory activity. For the ethanol–water fraction, the percentage inhibition values were 48.08% (100 ppm), 52.61% (200 ppm), 60.84% (400 ppm), 76.15% (800 ppm), and 97.74% (1600 ppm), yielding an IC_{50} value of 101.31 µg/mL, which indicates moderate anti-inflammatory activity. The percentage inhibition values for both fractions and the positive control were all above 20%, confirming their anti-inflammatory potential.

As stated by Hasim et al., (2019), samples with inhibition values greater than 20% are considered to have anti-inflammatory effects. Furthermore, the IC_{50} value of the ethanol–water fraction was lower than that of the ethyl acetate fraction, suggesting that the ethanol–water fraction exhibited a stronger anti-inflammatory effect. In general, a lower IC_{50} value indicates higher anti-inflammatory activity. The difference in IC_{50} values between the ethyl acetate and ethanol–water fractions may be influenced by several factors, including the altitude of the growing area, rainfall, and soil nutrient composition, all of which can affect the concentration of bioactive compounds and their pharmacological activities. For the positive control, sodium diclofenac, the percentage inhibition values were 54.75% (100 ppm), 57.86% (200 ppm), 64.82% (400 ppm), 74.78% (800 ppm), and 99.26% (1600 ppm), yielding an IC_{50} value of –70.85 µg/mL. This indicates that sodium diclofenac possesses very strong anti-inflammatory activity, as its IC_{50} value is below 50 µg/mL. The test concentrations ranging from 100 ppm to 1600 ppm were relatively high, given that the IC_{50} value was achieved around 70 ppm.

According to Wijayanti & Qomariyah, (2023), phytochemical screening of the ethanolic extract of cashew (Anacardium occidentale L.) leaves revealed the presence of several bioactive compounds, including alkaloids, flavonoids, saponins, triterpenoids, phenolics, and tannins in significant amounts. Flavonoids are believed to play a major role in anti-inflammatory activity by inhibiting the synthesis of prostaglandins from arachidonic acid (Costa, 2008). When the percentage inhibition value exceeds 20%, the extract is considered to possess anti-inflammatory potential. Tannins also contribute to anti-inflammatory activity due to their antioxidant properties, as they inhibit the production of O_2^-, thereby reducing the formation of H_2O_2, hypochlorous acid, and hydroxyl radicals (OH•). Moreover, steroid compounds are known to inhibit the production of inflammatory mediators such as leukotrienes, prostaglandins, histamine, and bradykinin.

The steroids exert anti-inflammatory effects by inhibiting the enzyme phospholipase A_2, thereby suppressing the formation of arachidonic acid — the substrate for lipoxygenase enzymes — and consequently blocking the release of inflammatory mediators. Various experimental techniques are used in the study of pharmacological, chemical, and herbal preparations to demonstrate anti-inflammatory effects, including the inhibition of oxidative phosphorylation (ATP biogenesis related to respiration), protein denaturation assays, erythrocyte membrane stabilization tests, lysosomal membrane stabilization tests, fibrinolytic assays, and thrombolytic aggregation studies (Bettiol et al., 2022).

Based on various studies, the chemical compounds responsible for anti-inflammatory activity are primarily flavonoids. Flavonoids inhibit the cyclooxygenase (COX) and lipoxygenase (LOX) enzymes and prevent leukocyte accumulation at the site of inflammation, thereby exerting anti-inflammatory effects. These compounds specifically suppress the formation and release of pro-inflammatory mediators produced during allergic or hypersensitivity reactions. Different subclasses of flavonoids exhibit varying degrees of efficacy in modulating inflammation. The anti-inflammatory mechanism of flavonoids can occur through multiple pathways, one of which involves the direct inhibition of COX and LOX enzyme activities, leading to the suppression of prostaglandin and leukotriene biosynthesis — the end products of the COX and LOX pathways.

Conclusion

Based on the results obtained in this study, both the ethyl acetate and ethanol–water fractions of cashew (*Anacardium occidentale* L.) stem bark demonstrated inhibition percentages above 20%, indicating potential anti-inflammatory properties. The IC_{50} values revealed that the ethanol–water fraction exhibited a lower IC_{50} compared to the ethyl acetate fraction, suggesting that the ethanol–water fraction possesses stronger anti-inflammatory activity than the ethyl acetate fraction.

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Author Contributions

Author contributions: J designed the study. SW and AWS conducted the experiments and performed data analysis. S contributed to statistical analysis, manuscript preparation and was responsible for research administration. All authors read and approved the final manuscript.

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Conflicts of Interest

The author declares that there are no conflicts of interest in the conduct or reporting of this research. All stages of the study titled In Vitro Anti-inflammatory Test of Cashew (*Anacardium occidentale* Linn) Stem Bark through Protein Denaturation Using a UV–Vis Spectrophotometer were carried out independently without interference from any external party. There are no financial, professional, or personal relationships that could influence the objectivity of the research results. The funding body had no role in the study design, data analysis, or publication decision.

References

Al-Khayri, J. M., Sahana, G. R., Nagella, P., Joseph, B. V., Alessa, F. M., & Al-Mssallem, M. Q. (2022). Flavonoids as Potential Anti-Inflammatory Molecules: A Review. *Molecules*, *27*(9), 2901. https://doi.org/10.3390/molecules27092901

Anggraeny, A. setyopuspito pramitaningastuti. E. N. (2017). Uji Efektivitas Anti Inflamasi Ekstrak Etanol Daun Srikaya (Annona Squamosa. L) Terhadap Udema Kaki Tikus Putih Jantan Galur Wistar. *Jurnal Ilmiah Farmasi*, *13*(1), 8–13.

Armadany, F. I., Wahyuni, W., Ardianti, M., & Mallarangeng, A. N. T. A. (2020). Uji Potensi Antiinflamasi Ekstrak Etanol Daun Bambu-Bambu (Polygonum pulchrum Blume) Dengan Metode Stabilisasi Membran Sel Darah Merah Secara In Vitro. *Majalah Farmasetika.*, *4*(1). https://doi.org/10.24198/mfarmasetika.v4i0.25873

Azzahra, F., Putri, V. M., Utami, A., & Astuti, F. (2024). Perbandingan Rendemen Dan Kandungan Kimia Ekstrak Daun Alpukat (Persea americana) Berdasarkan Perbedaan Metode Ekstraksi. *Jurnal Kesehatan Bakti Tunas Husada: Jurnal Ilmu-Ilmu Keperawatan, Analis Kesehatan Dan Farmasi*, *24*(2), 67–77. https://doi.org/10.36465/jkbth.v24i2.1301

Baldelli, A., & Aguilera, J. M. (2025). Size reduction and particle size influence the quantification of phenolic-type compounds and antioxidant activity in plant food matrices. *Trends in Food Science & Technology*, *162*, 105081. https://doi.org/10.1016/j.tifs.2025.105081

Bettiol, A., Galora, S., Argento, F. R., Fini, E., Emmi, G., Mattioli, I., … Becatti, M. (2022). Erythrocyte oxidative stress and thrombosis. *Expert Reviews in Molecular Medicine*, *24*, e31. https://doi.org/10.1017/erm.2022.25

Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., … Zhao, L. (2018). Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*, *9*(6), 7204–7218. https://doi.org/10.18632/oncotarget.23208

Choi, Ja Sung MD,. Kim, S.-W. (2025). Transplantation of Human Adipose Tissue Derived-SVF Enhance Liver Function in Fibrotic Liver Through High

Hepatogenic and Anti-Fibrotic Capabilities. *The American Journal of Gastroenterology*, *4*(8), 4119–4134. https://doi.org/10.14309/01.ajg.0000594000.09504.20

Ellita, T., Puspitasari, E., Wijayanti, T., & Harmastuti, N. (2025). Antioxidant Activity of Ethanol Extract, Fractions and Subfractions from Malay Apple Seed (Syzygium Malaccense). *East Asian Journal of Multidisciplinary Research (EAJMR)*, *4*(8), 4119–4134. https://doi.org/https://doi.org/10.55927/eajmr.v4i8.318

Esho, B. A., Samuel, B., Akinwunmi, K. F., & Oluyemi, W. M. (2021). Membrane Stabilization and Inhibition of Protein Denaturation as Mechanisms of the Anti-Inflammatory Activity of some Plant Species. *Trends in Pharmaceutical Sciences*, *7*(4), 269–278. https://doi.org/10.30476/TIPS.2021.93160.1118

Gelian, C., Nurlila, R. U., & Himaniarwati. (2024). Uji Aktivitas Fraksi Daun Pare (Momordica charantia) Terhadap Pertumbuhan Rambut Kelinci New Zealand White. *Jurnal Pharmacia Mandala Waluya*, *3*(3), 144–156. https://doi.org/10.54883/jpmw.v3i3.71

Gulo, K. N., Suhartomi, Saragih, A. D., Raif, M. A., & Ikhtiari, R. (2021). Antioxidant Activity of Flavonoid Compounds in Ethanol and Ethyl Acetate Extract from Citrus Sinensis. *2021 International Conference on Artificial Intelligence and Mechatronics Systems (AIMS)*, 1–6. IEEE. https://doi.org/10.1109/AIMS52415.2021.9466078

Harsini, H. (2017). Aktivitas antibakteri ekstrak etanolik kulit batang jambu mete (Anacardium occidentale Linn.) terhadap Staphylococcus aureus. *Majalah Kedokteran Gigi Indonesia*, *3*(3), 10. https://doi.org/10.22146/majkedgiind.17498

Hasim, H., Arifin, Y. Y., Andrianto, D., & Faridah, D. N. (2019). Ekstrak Etanol Daun Belimbing Wuluh (Averrhoa bilimbi) sebagai Antioksidan dan Antiinflamasi. *Jurnal Aplikasi Teknologi Pangan*, *8*(3), 86. https://doi.org/10.17728/jatp.4201

Hasyim, A., Suhaenah, A., & Tahir, M. (2023). Uji Aktivitas Antioksidan Fraksi Etil Asetat Daun Karet Kebo (Ficus elastica) Dengan Menggunakan Metode Frap. *Makassar Pharmaceutical Science Journal (MPSJ)*, *1*(3), 142–149. https://doi.org/10.33096/mpsj.v1i3.76

Hidayah, N. N., & Abidin, S. Z. (2017). The evolution of mineral processing in extraction of rare earth elements using solid-liquid extraction over liquid-liquid extraction: A review. *Minerals Engineering*, *112*, 103–113. https://doi.org/10.1016/j.mineng.2017.07.014

Jain, A. K., Singh, D., Dubey, K., Maurya, R., Mittal, S., & Pandey, A. K. (2018). Models and Methods for In Vitro Toxicity. In *In Vitro Toxicology* (pp. 45–65). Elsevier. https://doi.org/10.1016/B978-0-12-804667-8.00003-1

Khoury, D. S., Cromer, D., Reynaldi, A., Schlub, T. E., Wheatley, A. K., Juno, J. A., … Davenport, M. P. (2021). Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nature Medicine*, *27*(7), 1205–1211. https://doi.org/10.1038/s41591-021-01377-8

Kusumastuti, E., Handajani, J., & Susilowati, H. (2014). Ekspresi COX-2 dan Jumlah Neutrofil Fase Inflamasi pada Proses Penyembuhan Luka Setelah Pemberian Sistemik Ekstrak Etanolik Rosela (Hibiscus sabdariffa) (studi in vivo pada Tikus Wistar). *Majalah Kedokteran Gigi Indonesia*, *21*(1), 13. https://doi.org/10.22146/majkedgiind.8778

Mahran, R., Vello, N., Komulainen, A., Malakoutikhah, M., Härmä, H., & Kopra, K. (2023). Isothermal chemical denaturation assay for monitoring protein stability and inhibitor interactions. *Scientific Reports*, *13*(1), 1–12. https://doi.org/10.1038/s41598-023-46720-w

Mulyani, T., Setyahadi, S., & Wibowo, A. E. (2023). Uji Aktivitas Antiinflamasi Kombinasi Ekstrak Daun Torbangun (Plectranthus amboinicus (Lour.) Spreng.) dan Ekstrak Daun Kelor (Moringa oleifera Lam.) dengan Metode Penghambatan Denaturasi Protein. *PHARMACY: Jurnal Farmasi Indonesia (Pharmaceutical Journal of Indonesia)*, *20*(1), 26. https://doi.org/10.30595/pharmacy.v0i0.14246

Nirmala Aga, K. (2018). *Uji Kadar Flavonoid Total Ekstrak Etanol Kulit Batang Jambu Mete (Anacardium Occidentale Linn) Dengan Metode Spektrofotometri UV-VIS*. Poltekkes Kemenkes Kupang.

Novika, D. S., Ahsanunnisa, R., & Yani, D. F. (2021). Uji Aktivitas Antiinflamasi Ekstrak Etanol Daun Belimbing Wuluh (Averrhoa bilimbi L.) Terhadap Penghambatan Denaturasi Protein. *Stannum : Jurnal Sains Dan Terapan Kimia*, *3*(1), 16–22. https://doi.org/10.33019/jstk.v3i1.2117

Prayitno, S., Juwaeni, A., & Asri, A. (2022). Uji Efektivitas Anti Inflamasi Ekstrak Kulit Batang Jambu Mete (Anacordium occidentale) PADA Hewan Uji Mencit (Mus Musculus). *FitoMedicine:JournalPharmacyandSciences*, *12*(2), 102–110. https://doi.org/https://doi.org/10.47650/fito.v13i2.433

Rahmi, I. A., Jufri, M., & Mun'im, A. (2020). Extraction

of Quercetin from Nothopanax scutellarium Leaves via Ionic Liquid-based Microwave-assisted Extraction. *Pharmacognosy Journal*, *12*(6s), 1512–1517. https://doi.org/10.5530/pj.2020.12.207

Ramadhani, N., & Sumiwi, S. A. (2016). Aktivitas antiinflamasi berbagai tanaman diduga berasal dari flavonoid. *Farmaka*, *14*(2), 111–123. https://doi.org/https://doi.org/10.24198/jf.v14i2.10816

Roghini, R., & Vijayalakshmi, K. (2018). Phytochemical Screening, Quantitative Analysis of Flavonoids and Minerals in Ethanolic Extract of Citrus Paradisi. *International Journal of Pharmaceutical Sciences and Research*, *9*(11), 4859. https://doi.org/10.13040/IJPSR.0975-8232.9(11).4859-64

Samarakoon, M. and. (2023). In vitro Anti-Inflammatory Egg Albumin Denaturation Assay: An Enhanced Approach. *Journal of Natural & Ayurvedic Medicine*, *7*(3), 1–6. https://doi.org/10.23880/jonam-16000411

Siracusa, R., Fusco, R., Peritore, A., Cordaro, M., D'Amico, R., Genovese, T., … Impellizzeri, D. (2020). The Antioxidant and Anti-Inflammatory Properties of Anacardium occidentale L. Cashew Nuts in a Mouse Model of Colitis. *Nutrients*, *12*(3), 834. https://doi.org/10.3390/nu12030834

Vasques-Nóvoa, F., Angélico-Gonçalves, A., Alvarenga, J. M. G., Nobrega, J., Cerqueira, R. J., Mancio, J., … Roncon-Albuquerque, R. (2022). Myocardial oedema: pathophysiological basis and implications for the failing heart. *ESC Heart Failure*, *9*(2), 958–976. https://doi.org/10.1002/ehf2.13775

Veriony, L., Sudarsono, & Nugroho, A. E. (2011). Aktivitas Antiinflamasi Rebusan Kulit Batang Jambu Mete (Anacardium occidentale L.) Pada Udema Kaki Tikus Terinduksi Karagenin. *Majalah Obat Tradisional*, *16*(3), 145–152. https://doi.org/http://dx.doi.org/10.22146/tradmedj.8052

Wijayanti, A. R., & Qomariyah, N. (2023). Pengaruh Ekstrak Daun Mengkudu (Morinda Citrifolia L.) terhadap Kadar Hemoglobin dan Histopatologi Hepar Mencit Diabetes. *LenteraBio : Berkala Ilmiah Biologi*, *12*(1), 14–24. https://doi.org/10.26740/lenterabio.v12n1.p14-24

Wirawan, I. G. K. O., Aoetpah, A., & Jacob, J. M. (2021). Effectiveness Comparison Between Young Leaf Extracts Acacia nilotica with Desmanthus virgatus Against Vermicidal Potency of Haemonchus contortus In-vitro. *Jurnal Sain Veteriner*, *39*(2), 168. https://doi.org/10.22146/jsv.60809

Zeinali, M., Rezaee, S. A., & Hosseinzadeh, H. (2017). An overview on immunoregulatory and anti-inflammatory properties of chrysin and flavonoids substances. *Biomedicine & Pharmacotherapy*, *92*, 998–1009. https://doi.org/10.1016/j.biopha.2017.06.003