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Preclinical Study of n-Hexane Extract of Kapul Fruit (*Baccaurea macrocarpa*) as an Anti-Inflammatory

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Kapul;
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ABSTRACT

Plants of the genus *Baccaurea* have long been traditionally used in folk medicine. Previous studies reported that the n-hexane extract of kapul fruit (*Baccaurea macrocarpa*) exhibited extreme antioxidant activity with an IC_{50} value of 33.11 ppm. However, its potential anti-inflammatory activity has not been extensively investigated.

The results demonstrated that all three doses of the n-hexane extract exhibited anti-inflammatory activity, as indicated by a reduction in edema volume. Among the tested doses, 600 mg/kg BW was the most effective ini decreasing edema volume. Statistical analysis using one-way ANOVA showed a significance value of 0.000, confirming a significant difference among the five treatment groups. Furthermore, post hoc analysis indicated that dose III did not differ significantly from the positive control but showed a significant difference compared to the negative control. Conversely, doses I and II exhibited significant differences when compared to both the positive and negative controls.

These findings suggest that the *n*-hexane extract of *B. macrocarpa* fruit possesses anti-inflammatory activity, with the 600 mg/kg BW dose demonstrating the most potent effect, comparable to that of diclofenac sodium.

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Introduction

Inflammation is a local protective response triggered by tissue damage caused by physical trauma, harmful chemicals, or microbiological agents. Inflammation is the body's natural defense mechanism in response to injury or tissue damage caused by physical trauma, exposure to harmful chemicals, or microbial infection. This condition is usually characterized by classic symptoms such as redness, heat, swelling (edema), pain, and impaired tissue function. Inflammation responds to tissue damage by bringing defense cells and molecules from the bloodstream to the location needed to eliminate the offending agent. The inflammatory reaction in the body serves to destroy and reduce the injurious agent and injured tissue, as well as prepare the tissue for the healing process (Suryandari et al., 2021). Commonly used anti-inflammatory drugs include steroidal anti-inflammatory drugs and nonsteroidal anti-inflammatory drugs (Widiyantoro et al., 2012). However, both classes have numerous side effects. Steroidal anti-inflammatories can cause peptic ulcers, decreased immunity to infection, and osteoporosis, while nonsteroidal anti-inflammatories cause gastric ulcers, bleeding, kidney disorders, and anemia (Rinayanti et al., 2014).

One plant with potential anti-inflammatory effects is the Baccaurea genus. This genus belongs to the Phyllanthacea family and comprises approximately 43 species widely distributed from India, Kalimantan, Sumatra, Java, Peninsular Malaysia, Thailand, the Philippines, and the Pacific Islands. Baccaurea fruit is generally consumed fresh, while wood is used as a building material. This genus contains secondary metabolites with important biological activities, such as antioxidant, anticancer, antimicrobial, antidiabetic, antitrypanosomal, and anti-inflammatory properties (Gunawan et al., 2016).

Baccaurea macrocarpa, better known as jantikan, kapul, or tampoi, is reported to contain secondary metabolites in its fruit, including flavonoids, steroids, and terpenoids (Madiyawati et al., 2017). Flavonoids are known to act as anti-inflammatories through inhibiting cyclooxygenase and lipoxygenase enzymes and suppressing leukocyte accumulation in areas of inflammation (Agustina et al., 2015). Previous research also showed that the n-hexane extract of kapul fruit has antioxidant activity with an IC50 value of 33.11 ppm, which is considered a powerful antioxidant. Based on this background, kapul fruit has excellent potential to be developed as an anti-inflammatory agent, and research related to kapul fruit as an anti-inflammatory is needed.

Method

Research Design

This research is an experimental study using test animals. The test animals used were male white Wistar rats (Rattus novergicus) aged 2-3 months and weighing 200-300 g. This study has obtained ethical approval from the Faculty of Medicine and Health Sciences Ethics Committee, Lambung Mangkurat University, under number 115/KEPK-FK UNLAM/EC/IV/2020.

Extract Preparation

The sample was Kapul Fruit (*Baccaurea macrocarpa*) taken in the Rantau Bujur Village area, Aranio District, Banjar Regency. Determination under number 192c/LB.LABDASAR/XI/2019 was conducted at the Basic Laboratory of the Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University. Kapul fruit was washed and separated between the skin and the flesh, then dried using an oven at a temperature of 60°C. The dried fruit was then ground and sieved using a 40 mesh sieve. Extraction was carried out by the soxhletation method using 125 mL of n-hexane solvent and 25 g of kapul fruit powder at a temperature of 50°C until the cycle drops were no longer colorless. The liquid extract was evaporated to obtain a thick extract (Rosita et al., 2017).

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Phytochemical Screening

Saponin

The extract was suspended in warm water, shaken, and allowed to stand for 10 minutes. A positive result was indicated by the formation of foam that persisted for 10 minutes and persisted after the addition of HCl (Nugrahani et al., 2016; Nurviana, 2016; Yunita et al., 2009).

Flavonoid

The extract was reacted with Mg powder, concentrated HCl, and amyl alcohol, then shaken and allowed to separate. A positive result was indicated by the formation of a reddish-brown color in the amyl alcohol layer (Nugrahani et al., 2016; Yunita et al., 2009).

Alkaloid

Ammonia and chloroform were added to the extract, which was then heated, agitated, and filtered. A solution of 2N H_2SO_4 was added and shook. Dragendorff's, Wegner's, and Mayer's reagents were used to test the upper portion of the extract. According to Nugrahani et al. (2016) and Yunita et al. (2009), the presence of a white precipitate with Mayer's reagent, an orange-red precipitation with Dragendorff's reagent, and a brown precipitate with Wegner's reagent all suggested a successful outcome.

Steroids/Triterpenoids

The extract is mixed with chloroform, then filtered, and two drops of acetic anhydride and concentrated $H2SO_4$ are added. A bluish-green color indicates a positive steroid presence, and a purple color indicates a positive triterpenoid presence (Simaremare, 2014).

Taninns

The thick extract is mixed with a 1% gelatin solution in NaCl. The formation of a white precipitate indicates a positive result (Tiwari et al., 2011).

Phenols

The thick extract is added to the $FeCl_3$ solution. A positive result is indicated by the formation of a bluish-green black color (Tiwari et al., 2011).

Antiinflammatory Activity

Twenty-five mice were divided into five treatment groups. The negative control group received 1% Na-CMC, the positive control group received 6.75 mg/kgBW of sodium diclofenac, and three treatment groups received 200, 400, and 600 mg/kgBW of n-hexane extract of kapul fruit. Each mouse was marked at the ankle, which was then measured using a plethysmometer and recorded as the initial volume (V_0). Each mouse was then given an oral suspension of the test material according to its respective group. One hour after treatment, the mice were given a subplantar injection of 0.1 mL of 1% carrageenan. Edema volume was measured every hour for 6 hours after the induction (Vt). Edema volume (Vu) represents the difference in the size of the mouse's paw before and after inflammation. Quantitative data for the study were AUC (Area Under the Curve), which is the average edema curve versus time, and the percentage of anti-inflammatory effect. Based on the AUC value data, the % anti-inflammatory power can be obtained using the formula (Sujono et al., 2012) :

$$\% \text{ inflammatory strength} = \frac{AUCk - AUCp}{AUCk} \times 100\%$$

Description:
AUCk : AUC of the average edema volume curve versus time for the negative control
AUCp : AUC of the edema volume curve versus time for the treatment group/test animal

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Data Analysis

The research data was analyzed using SPSS software. Data normality was tested using the Shapiro-Wilk test; a sig value > 0.05 usually indicates distributed data. Homogeneity was then tested using Levene's; a sig value > 0.05 indicates homogeneous data. Statistical analysis was then performed using ANOVA with a 95% confidence level. Finally, an LSD test was performed to identify which groups differed from the others.

Results and Discussion

The yield obtained from the extraction process using the soxhletation method was 5.00%. Based on research by Wijaya et al. (2022), extraction using the soxhletation method yielded a higher yield than the maceration method. Soxhletation is a technique for separating active compounds from a mixture by heating. The solvent is repeatedly moved in a cycle, allowing the sample to interact with the pure solvent, optimizing the extraction process without requiring large solvent volumes (Anam et al., 2014; Irianty & Yenti, 2014).

Table 1. Phytochemical Screening Results of n-Hexane Extract of Kapul Fruit

Number	Secondary Metabolites	Results
1	Saponin	+
2	Flavonoid	+
3	Alkaloid	-
4	Steroids/triterpenoids	+
5	Taninns	-
6	Phenols	+

Keterangan: (+) Identified, (-) Not Identified

The results of phytochemical screening on n-hexane extract of kapul fruit showed positive results containing saponins, flavonoids, steroids, triterpenoids, and phenols. The foam formed in the saponin test occurs due to glycoside compounds that can form foam in water and undergo hydrolysis into glucose and other compounds (Agustina et al., 2017). A positive flavonoid test produces an orange color because magnesium reduces the carbonyl group in the benzopyrone core of the flavonoid structure, which then reacts with HCl to form an orange flavylium salt (Bawekes et al., 2023). The results of the steroid test show a purple color and steroids a green color due to acetic acid and sulfuric acid binding to steroid and triterpenoid compounds (Agustina et al., 2017). A greenish-black color change indicates a positive result in the phenol test because $FeCl_3$ reacts with the hydroxyl group bound to the unsaturated carbon in the phenol compound so that it can produce a greenish-black complex compound and the role is played by the Fe^{3+} ion which undergoes hybridization (Bawekes et al., 2023).

Carrageenan is a polysaccharide derivative that the body recognizes as a foreign substance (Novadyanti, 2015). Carrageenan can cause cell damage, triggering the release of inflammatory mediators, such as histamine, serotonin, bradykinin, and prostaglandins. This mediator release plays a role in acute inflammation, characterized by the appearance of edema. Carrageenan-induced edema lasts 6 hours and gradually resolves over 24 hours (Ermawati & Nurmila, 2019).

The anti-inflammatory activity of n-hexane extract of kapul fruit was tested using the edema method on rat paws, using a plethysmometer filled with mercury. The working principle of the plethysmometer is based on Archimedes' Principle, which explains that when an object is immersed in a liquid, an upward force or pressure is generated (Sukmawati et al., 2015).

The results of observations on the volume of edema in the rat's paws showed that the negative control group was the group that experienced the most enormous edema volume compared to the positive control group, extract dose 200 mg/kgBW, extract dose 400 mg/kgBW and extract dose 600

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mg/kgBW. This is likely because the administration of Na-CMC to the negative control group did not affect reducing edema volume. Meanwhile, in the positive control group that received sodium diclofenac, showed that the peak edema volume occurred at the 4th hour, as well as in the extract dose 400 mg/kgBW and extract dose 600 mg/kgBW groups, and in the extract dose 200 mg/kgBW group, the peak edema occurred at the 5th hour. This increase in edema volume is due to the release of inflammatory mediators in the tissue after the rats were induced by carrageenan. The decrease in edema volume in the positive control group, the 200 mg/kgBW extract, the 400 mg/kgBW extract, and the 600 mg/kgBW extract occurred due to inhibition of prostaglandin synthesis in the tissue (Apridamayanti et al., 2018).

The Area Under Curve (AUC) describes the volume of edema in each group per unit of time. A higher AUC value indicates a lower anti-inflammatory activity of the drug in reducing edema volume. Conversely, a lower AUC value indicates a greater anti-inflammatory activity in reducing edema volume (Pramitaningastuti & Anggraeny, 2017). Table 2 shows the total AUC values in the treatment groups can be seen in Table 2.

Table 2. Total AUC Calculation Results

Number	Groups	Total AUC
1	Negative control (Na-CMC 1%)	0,073 ± 0,0057
2	Positive control (Na-Diklofenak 1,35 mg/200 gBB)	0,028 ± 0,0042*
3	Extract dose 200 mg/kgBW	0,045 ± 0,0051**
4	Extract dose 400 mg/kgBW	0,037 ± 0,0047**
5	Extract dose 600 mg/kgBW	0,033 ± 0,0053*

Note:
*Significantly different from negative control
**Significantly different from negative and positive controls

Based on the results in Table 2, it can be seen that the AUC values in order from largest to smallest are found in the negative control group, extract dose 200 mg/kgBW, extract dose 400 mg/kgBW, extract dose 600 mg/kgBW, and the positive control. The statistical results found that the 600 mg/kgBW dose group had an AUC value significantly different from the negative control and not significantly different from the positive control. Meanwhile, the AUC values of the 200 mg/kgBW and 400 mg/kgBW extract groups significantly differed from those of the negative and positive control groups.

Table 3. Results of the Anti-inflammatory Power Test Calculation

Number	Groups	% Anti-inflammatory strength
1	Negative control (Na-CMC 1%)	0 %
2	Positive control (Na-Diklofenak 1,35 mg/200 gBB)	61,64 %
3	Extract dose 200 mg/kgBW	38,63 %
4	Extract dose 400 mg/kgBW	49,04 %
5	Extract dose 600 mg/kgBW	54,79 %

Based on the results in Table 3, the highest to lowest anti-inflammatory power, respectively, are the positive control group, extract dose 600 mg/kgBW, extract dose 400 mg/kgBW, extract dose 200 mg/kgBW, and the negative control. From this, it can be seen that the treatment group that has the most effective potential to inhibit inflammation in the n-hexane extract of kapul fruit is a dose of 600 mg/kgBW, but it does not have better anti-inflammatory activity than sodium diclofenac. The study's results on the anti-inflammatory activity of the n-hexane extract of kapul fruit show that the increasing dose of the extract also increases the percentage of anti-inflammatory power or anti-inflammatory activity. The existence of anti-inflammatory activity is thought to be due to the presence

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of compounds of the saponin, flavonoid, steroid/triterpenoid, and phenol groups contained in the n-hexane extract of kapul fruit.

Conclusions and Suggestions

The n-hexane extract of kapul fruit (Baccaurea macrocarpa) has been shown to contain saponins, flavonoids, steroids, triterpenoids, and phenols. The n-hexane extract of kapul fruit (Baccaurea macrocarpa) has anti-inflammatory activity at 200, 400, and 600 mg/kgBW doses. Further research can be carried out on fractionation to identify the metabolites responsible for the anti-inflammatory activity.

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