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Evaluation of Antioxidant and Anti-Inflammatory Activities of Seed Extract of *Heritiera littoralis*

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Abstract: Plant bioactive compounds are typically distributed across leaves, stems, and fruits. The Dungun plant (*Heritiera littoralis*), a mangrove species, is a promising source of bioactive compounds. However, research on the bioactive properties of *H. littoralis* seeds remains limited and warrants further investigation. This study aimed to isolate and characterize the bioactive compounds from *H. littoralis* seed extracts for their antioxidant and anti-inflammatory potential, with the aim of developing functional food applications. The research methodology involved the extraction and fractionation of *H. littoralis* seeds, antioxidant analysis using the DPPH assay, in vivo evaluation of anti-inflammatory activity using a rat paw edema model, and in silico molecular docking studies. Ethanol extraction yielded secondary metabolites including flavonoids, tannins, saponins, and steroids. Fractionation using Vacuum Liquid Chromatography (VLC) produced three main fractions. Fraction F2 was further purified by Gravity Column Chromatography (GCC), resulting in a pure isolate (F2.3).

The melting point of the isolate (175–177°C) was consistent with that of catechins, suggesting its high purity. Characterization of the isolate using UV-Vis spectrophotometry revealed two absorption peaks at 218 nm and 280 nm, indicative of hydroxyl groups and electron transitions from the carbonyl groups. FTIR analysis supported this identification, with absorption bands at 3346.55 cm^{-1} (-OH group), 2925.67 cm^{-1} (C-H alkane), and 1521.35 cm^{-1} (C=C aromatic), confirming the isolate's similarity to catechin. The anti-inflammatory activity was assessed using a rat paw edema model. The results showed that the highest inhibition percentage was achieved with Na diclofenac (80.21%), followed by a 15 mg/kg body weight (BW) extract (62.63%), and a 10 mg/kg BW isolate (53.13%). Antioxidant activity testing via the DPPH method revealed that the ethanol fraction exhibited significant activity, with an IC_{50} value of 26.51 ppm, compared to ascorbic acid (positive control) with an IC_{50} of 5.68 ppm. These findings demonstrate that *H. littoralis* seeds have substantial potential as sources of effective antioxidant and anti-inflammatory agents, highlighting their potential for future development in functional foods and therapeutic applications.

Keywords: Antioxidant, Anti-Inflammatory, Bioactive Compouds, Heritoria littoralis.

*濱海黑藻*種子萃取物的抗氧化和抗發炎活性評價

摘要：植物的生物活性化合物通常分佈於葉、莖和果實中。海欖仁（濱海黑藻）是一類富含生物活性化合物的紅樹植物。對於海欖仁果實中生物活性化合物的研究仍然很少，亟需進一步探索。本研究旨在從海欖仁果實萃取中獲取具有抗氧化和抗發炎活性的生物活性化合物，並探討其作為功能性食品的發展潛力。研究方法包括對海欖仁果實進行萃取與分餾，採用DPPH法測試抗氧化性，對小鼠水腫進行抗發炎活性體內測試，以及進行分子對接的電腦模擬研究。使用乙醇作為溶劑萃取海欖仁種子，並獲得次級代謝物如黃酮類、單寧、皂苷和類固醇。真空液相層析分餾產生三個主要部分 進一步以重力柱層析分離F2部分，獲得純淨的F2.3分離物，其熔點測試顯示其接近兒茶素的化學特性（175-177° C，表明 該分離物 具有高純度。利用UV-Vis分光光度計對該分離物進行表徵，顯示在218nm和280 nm 處具有兩 個吸收峰，表示存在羥基和羰基的電子轉移。FTIR分析顯示在3346.55 cm^{-1}（-OH基)2925.67 cm^{-1}（C-H烷基)和 1521.35 cm^{-1}（C=C）芳香基)處有吸收，支持該分離物為兒茶素的假設。通過大鼠足部水腫模型進行抗發炎測試顯示，Na雙氯芬酸的抑制率最高（80.21%），其次是15mg/KgBW萃取物（62.63%）和10mg/KgBW的分離物（53.13%）。此外，使用DPPH法的抗氧化活性測試表明，乙醇部分的抗氧化活性顯著，IC_{50}為26.51ppm，而作為陽性對照的抗壞血酸的IC_{50}為5.68ppm。這些結果表明，海欖仁種子具有作為高效抗氧化和抗發炎劑的潛力

关键词：抗氧化劑、抗發炎劑、生物活性化合物、*濱海黑藻*

1. Introduction

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are free radicals that disrupt metabolic processes and compromise overall health [1,2]. Their overproduction leads to oxidative stress, which is closely associated with various physiological dysfunctions [3]. The interplay between oxidative stress and inflammatory pathways is pivotal for the initiation and progression of numerous diseases [4,5]. During inflammation, the release of mast cell proteases initiates a cascade of redox events that is mediated by free radicals. These events are essential for cellular signalling and pathogen defence mechanisms [6]. However, excessive inflammation can compromise the body's innate immune response and is strongly linked to oxidative stress, contributing to the pathogenesis of chronic diseases, such as cancer, diabetes, hypertension, septic shock, asthma, arthritis, atherosclerosis, Parkinson's disease, and Alzheimer's disease [7,8]. Given the critical role of inflammation in these conditions, strategies aimed at regulating inflammatory and oxidative processes represent promising avenues for mitigating or preventing major neurodegenerative and chronic disorders [9].

Compounds with antioxidant activity play a crucial role in modulating the body system by neutralizing free radicals, thereby reducing oxidative damage [10].

Antioxidants mitigate the production of reactive oxygen species (ROS) and free radicals, which in turn lower the risk of lipid peroxidation, oxidative stress, post-translational protein modifications, and DNA damage. Although synthetic antioxidants are commonly utilized as treatments, they are often expensive and may cause adverse side effects. As a result, there is a growing interest in plant-derived antioxidants, which are more affordable, have fewer side effects, and are widely accessible [9,10]. Medicinal plants are emerging as promising sources of antioxidant and anti-inflammatory agents, offering the potential to strengthen the body's innate immune system against free radical-induced inflammation [11,12]. The exploration of natural extracts with dual antioxidant and anti-inflammatory properties is gaining attention as a preventive and therapeutic strategy to address oxidative stress and inflammation-related diseases.

Jambi Province, with its rich biodiversity, harbors numerous traditional medicinal plants that remain underexplored [13]. Among these, mangrove species have a significant potential for further investigation. Mangroves are abundant along the eastern coast of Tanjung Jabung, Jambi, and include species such as *Sonneratia alba* [14,15], *Acanthus ilicifolius* [16], *Avicennia marina* [17,18], *Rhizophora apiculata*, *Bruguiera gymnorhiza*, and *Heritiera littoralis* [14,19]. These species have been reported to exhibit a range of bioactivities, including antimicrobial, antioxidant, anti-inflammatory, and cytotoxic effects. The vast diversity of mangrove species and their bioactive potential underscores the need for further exploration to harness their medicinal benefits. This could pave the way for the development of cost-effective natural alternatives to synthetic antioxidants, contributing to improved healthcare solutions.

Heritiera littoralis (commonly known as Dungun) is a mangrove species with a substantial potential for further exploration is *Heritiera littoralis* (commonly known as Dungun). This validated mangrove species contains a diverse range of chemical compounds distributed across its roots, stems, leaves, and fruits. Traditionally, *H. littoralis* has been used to treat various ailments, including diarrhea, digestive disorders, and dysentery [20], as well as skin conditions such as dermatitis, rashes, eczema, boils, itching, scabies, wounds, infections, and liver diseases [21].

The bark of *H. littoralis* has demonstrated anti-inflammatory activity by inhibiting the release of nitric oxide (NO) and cyclooxygenase-2 (COX-2) enzyme [19]. Further phytochemical studies have revealed that *H. littoralis* contains triterpenoids, flavonoids, and phenylpropanoid-glycerol compounds [22]. The leaves contain terpenoids, steroids, tannins, proanthocyanidins, and anthraquinones. At the same time, its roots contain flavonoids, sesquiterpenes, terpenoids, and astilbin, which are traditionally used to treat oral infections and toothaches [20]. These bioactive compounds exhibit significant anti-inflammatory properties by inhibiting cyclooxygenase and lipoxygenase enzymes and preventing leukocyte accumulation in the bloodstream [23,24].

Although extensive research has been conducted on the leaves and bark of *H. littoralis*, the fruits still need to be studied despite their potential as a source of bioactive compounds. The fruits are believed to contain secondary metabolites, such as flavonoids, terpenoids, steroids, tannins, and anthraquinones [25], key plant metabolism products. Traditionally, Dungun fruit has been consumed for its anti-inflammatory, antibacterial, blood sugar-lowering, detoxifying, and anticoagulant properties. Their antioxidant activity, which neutralizes free radicals and mitigates oxidative stress-induced damage, makes them intriguing targets for further investigation [26].

Previous research suggests that seed extracts often exhibit greater bioactivity than other plant parts such as leaves and bark [26]. For instance, the fruit extract of *Clusiaceae* species has been reported to contain higher antioxidant levels than the leaves [27]. Conversely, the fruit extract of *Ziziphus spina* contains higher concentrations of bioactive compounds and exhibits stronger antibiotic activity [28]. Similarly, in mangrove species, fruit extracts of *Avicennia marina* have been shown to possess higher total phenolic, flavonoid, and antioxidant contents than their stems, leaves, and roots [17]. The fruits of *Sonneratia alba* also contain elevated levels of total phenolics, proteins, and antioxidants compared to their roots and leaves [15].

Given these observations, *H. littoralis* fruits represent a compelling subject for further exploration. Despite its potential, research on the fruits of this species remains limited, particularly regarding crude extracts and detailed phytochemical analyses. Expanding the focus of studies to include the fruits of *H. littoralis* could reveal valuable bioactive compounds with significant antioxidant and therapeutic potential.

2. Materials and Methods

2.1. Materials and Instrumentations

The materials used in this study were *H. littoralis* seeds obtained from Kawasan Mangrove in West Tanjung Jabung dan East Tanjung Jabung, Provinsi Jambi, Indonesia Chemical were used Ethanol, Folin-Ciocelteu, Na_2CO_3 7%, Methanol (p.a), Gallic acid, $AlCl_3$, Sodium Acetate, Quarcetine, ascorbic acid, DPPH ((1,1-Diphenyl-1-picrylhydrazyl) (Sigma-Aldrich, Singapore). A UV-Vis spectrophotometric spectrophotometer (Thermo-Fisher Orion Scientific AQ8100, Waltham, MA, USA) with a wavelength of 200-800 nm and an FTIR spectrophotometer (Thermo Fisher Scientific) at wavenumbers of 4000-400 cm^{-1}.

2.2. Methods

2.1.1. Sample Preparation

The samples were then shorted, washed, and aerated. The samples were then mashed and filtered through a 60-mesh sieve to obtain Simplisia powder. Simplisia was extracted with ethanol (70% v/v) (ratio 1:10) by maceration for 2 × 24 h. The filtrate was filtered through Whatman No.1 and evaporated using a rotary vacuum evaporator (Buchi) at 50°C to obtain the crude extract [29].

2.2.2. Extraction and Fractination

The extraction was performed using the maceration method. A total of 300 g of *H. littoralis* seed simplicia was dissolved in 1.5 L of 70% ethanol for 3 × 24 h (duplo). The extract was fractionated using three solvents: n-hexane, ethyl acetate, and ethanol (analytical grade). The fractions were evaporated using a vacuum rotary evaporator (Buchi).

Extract Yield

The yield was calculated according to previous studies [8] using the following formula 1.

$$\%\text{Yield Extract} = WE/WS \times 100\% \quad (1)$$

Notes
WE = Weight of Extract (g)
WS = Weight of Simplicia (g)

2.2.3. Bioactive Isolation and Phytochemical Screening

The ethanol fraction was separated using vacuum liquid chromatography. Subsequently, fractions showing distinct spots were separated using gravity column chromatography. The stationary phase used in vacuum liquid column chromatography was silica gel G60 with a mesh size of approximately 200, while the mobile phase was chosen based on the optimal TLC results using eluents of 100% n-hexane, n-hexane: acetate, 100% ethyl acetate, and ethyl acetate: ethanol. Fraction F.2 was selected for further analysis owing to its superior antioxidant activity. Phytochemical screening was conducted to identify the groups of flavonoids, phenolics, steroids, alkaloids, tannins, and saponins present in the extracts and fractions, following procedures outlined in previous studies [30].

2.2.4. Antioxidant Activity (In Vitro)

The antioxidant activity was analyzed using the DPPH method. The analyzed samples included extracts and fractions at varying concentrations (0, 10, 25, 50, and 100 ppm). DPPH radical scavenging analysis was conducted using UV-Vis spectroscopy at a wavelength of 517 nm. The percentage of DPPH inhibition was analyzed to determine the IC_{50} value. The DPPH inhibition curve was created by plotting the% inhibition against the logarithm of the sample concentration. Linear regression was performed, $y = ax + b$, and IC_{50} was calculated using Equation 2.

$$IC_{50} = (50 - b)/a \quad (2)$$

y: % inhibition; x: log of concentration; m: slope; c: intercept.

2.2.5. Anti-Inflammatory Activities

Thirty mice were devided into five groups (Table 1). Male mice (weighing 20–25 g, aged 7-8 weeks) were acclimatized for 18 h. The initial paw volume of the mice was measured (V_o), followed by a 1 % carrageenan injection (0.3 mL) subplantarly. Paw volume was measured every 30 min over a 180 min observation period (Vt). The anti-inflammatory activity was assessed by comparing the differences between Vt and Vo.

Table 1. Sample Codes

No	Samples	Information
1	C-	Negative Control Na-CMC 1%
2	C+	Positive Control; Natrium Diklofenac
3	CE	Crude extract ethanol
4	HeF	n-hexane fraction
5	EaF	Ethyl acetate fraction
3	EF	Ethanol fraction 15 mg/KgBW
4	IE	Isolate of Ethanol

The data obtained in the form of rat foot volume were used to calculate the volume of edema. The volume of edema is the difference between the feet of rats before and after inflammation. The equation for calculating the edema is as formula 3 [16]:

$$\%\ \text{Edema} = (Vt\text{-}V_0)/V_0 \times 100\% \quad (3)$$

Description:
Vt: volume of rat paw after being induced with 1% carrageenan at time t.
VO: volume of rat's paw before being induced with 1% carrageenan.

The percentage of anti-inflammatory power (edema volume inhibition) was calculated based on the percentage reduction in edema using Formula 4.

$$\%\ \text{Inflammation Inhibition} = (AUCk - AUCp)/AUCk \times 100\% \quad (4)$$

Description:
AUCk: AUC of average edema volume curve against time for negative control
AUCp: AUC of edema volume curve against time for treatment group in each individual

2.3. Statistical analysis

Data were analyzed using Excel and GraphPad Prism for Windows version 5 (Graph Pad Software Inc., San Diego, CA, USA) using one-way ANOVA. Statistical significance was set at p value < 0.05. All measurements were performed in triplicate, and the results are presented as the mean ± standard deviation.

2.4. Molecular Docking

Three bioactive compounds, flavan-3,6,7,4',5'-pentaol, catechin, and eriodictyol, were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The target protein receptor, COX-1 (prostaglandin H2 synthase-1), was retrieved from the Protein Data Bank (PDB) under ID code 1EQG (https://www.rcsb.org/. Ligand preparation was conducted using Chem3D version 22.0, Discovery Studio 2021, and ligand optimization was performed using the AutoDockTools application. Similarly, the three-dimensional macromolecular structure of COX-1 was optimized using AutoDockTools to ensure compatibility with docking analysis. Molecular docking was performed using PyRx version 0.8, employing the AutoDock Vina algorithm to assess the binding interactions between the ligands and the receptor. The docking results were visualized and analyzed using Discovery Studio Visualizer 2021 to facilitate the interpretation of molecular interactions and binding affinities.

3. Result and Discussion

The extract yield was determined by comparing the extract obtained with the initial simplicia. The extract obtained was reddish-brown and evaporated using a Rotary Evaporator. The amount of extract and % yield from the extracted evaporation results are presented in Table 2.

Table 2. Extract and % Yield

Samples	Simplicia (g)	Crude (gr)	Yield (%)
CE	300 g	48 g	16
HeF	40 g	3.59 g	8.83
EaF	40 g	5.73 g	14.10
EtF	40 g	15.61 g	38.43

Table 3. Phytochemical Screening Fractions

Secondary Metabolites	CE	HeF	EaF	EtF
Alkaloids	-	-	-	-
Flavonoids	+	-	-	+
Saponin	+	+	+	-
Tanin	+	+	+	+
Steroids	+	-	-	+
Triterpeoid	-	-	-	-

3.1. Isolation Bioactive Compounds

The ethanol fraction contained several groups of secondary metabolites. Separation was performed using Vacuum Liquid Chromatography (VLC) (Figure 1). Three fractions were obtained: F1 (0.4 g), F2 (9.8 g), and F3 (0.6 g) (Table 4), F1 contains bioactive compound flavonoids. F2 contained flavonoids, saponins, and tannins, whereas F3 contained flavonoids and tannins. Additionally, isolation of F2 yielded I2, which predominantly contained tannins (Table 5).

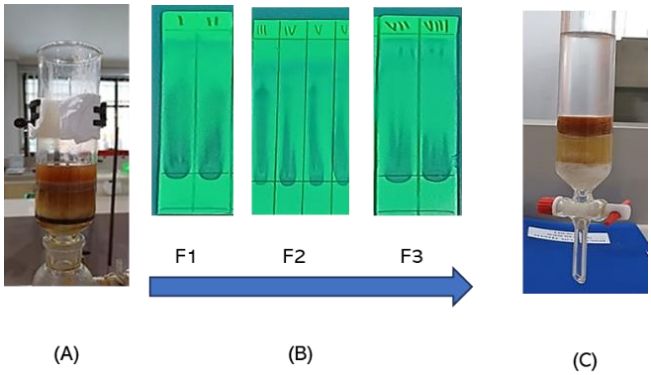


Figure 1. Isolation bioactive compounds; (A0) Vacuum Liquid Chromatography; (B) Spot pattern of Thin Layer Chromatography; (C). Gravity Column Chromatography

Table 4. Fractions Weight

Fractions	Vials	Weight(gr)
EF1	1-2	0.4 gr
EF2	3-6	9.8 gr
EF3	7-8	0.6 gr

Table 5. Phytochemical Screening of Fractions

Secondary Metabolites	EF1	EF2	EF3	IE
Alkaloids	-	-	-	-
Flavonoids	+	+	+	-
Saponin	-	+	-	-
Tanin	-	+	+	+
Steroids	-	-	-	-
Triterpeoid	-	-	-	-

Notes: EF1 = Fraction 1, EF2 = Fraction 2, EF3 = Fraction 3, IE = Isolate from Fraction 2.

3.2. Isolate Characterization

UV-Vis spectroscopy was used to analyze the structural and chemical properties of the bioactive compounds. This technique measures the absorbance of ultraviolet and visible light by using a compound. An IR spectrophotometer was used to determine the functional groups present in the isolate. The results of identifying the isolate using the IR spectrophotometer obtained several wavenumber spectra that were identical to the wavenumber spectrum of tannin (Figure 2 and Figure 3). Data for the comparison of the wavenumber spectrum between the isolate and the tannin compound are shown

in Table 6. The wave numbers of the isolate that are identical to the wave numbers of tannin include wave number 3346.55 cm-1, which indicates the stretching vibration of the hydroxyl group (-OH), and 2925.67, which indicates the stretching of the C-H alkane group. Wave number 1521.35 cm^{-1}, indicating the presence of an aromatic C = C range. Wave number 1040.92 cm-1 primary alcohol C-O group [31,32].

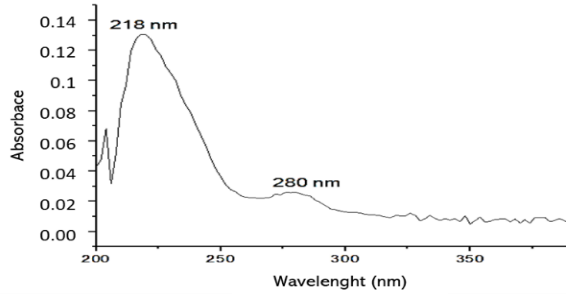


Figure 2. UV-vis spectrum of Isolate

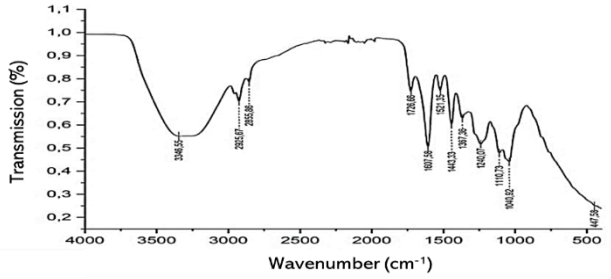


Figure 3. IR spectrum of Isolate

Similar results were also found in a previous study [33] that the catechin spectrum can show strong absorption around 3500 and 3000 cm^{-1} with wide and strong bands centered at 3317.9 cm^{-1} which has a type of hydroxyl group bond (OH) due to the variety of hydrogen bonds between OH. In this spectrum, a sharp peak can be seen at 2929.43 cm^{-1} associated with symmetric and antisymmetric -C-H- stretching vibrations in the CH_2 and CH_3 groups.

The absorption at 1607.58 indicates the presence of C=C vibrations and aromatic C=C at a wave number of 1521.35 cm^{-1}. The absorption at 1240.07 indicates C-H vibrations. From FTIR spectrophotometric characterization, it can be seen that the isolate is suspected to contain catechin or flavan-3ol-type tannin compounds (Figure 4). The catechin standard has an absorption peak at 3000-3700 cm-1 (3306), which corresponds to the O-H group (hydroxyl), the C=C group (1607), and the C-O group (1017).

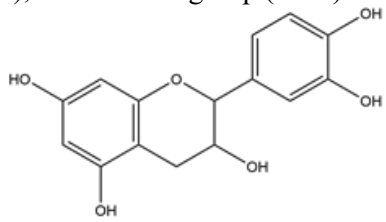


Figure 4. Chemical structure of catechin

Table 6. IR-Spectrum

Peaks	Wavenumber (cm^{-1})		Wavenumber Ranges (cm^{-1})	Type Bond	Functional Groups
	Isolate	Cathecine [34]			
1	3346.55	3364.52	3500-3000	O-H	Hydroxyl
2	2925.67	2925.53	3000-2900	C-H	CH_2 saturation groups
3	1607.58	1617.92	1450–1600	C=C	Flavonoid and Aromatic Rings
4	1521.35	1524.01	1645-1615	C=C Aromatic	Flavonoid and Aromatic Rings
5	1367.36	1351.53	1515-1560	C-H	Favonoid CH_3
6	1240.07	1207.99	1345-1385	C-H	Flavonoid CH_3
7	1040.92	1043.80	1280-1220 1120-1080	C-O	Primary alcohol

3.3. Antioxidant Activities

The antioxidant activity was tested using the DPPH (2,2-diphenyl-1-ficrhydrazyl) method. The principle of measuring antioxidant activity using the DPPH method is based on the intensity of the purple DPPH color, which is proportional to the concentration of the DPPH solution. DPPH free radicals with unpaired electrons make the solution purple. The purple color changed to yellow when the electrons were paired. Color changes occur when free radicals are dampened by the reaction between DPPH molecules and the hydrogen atoms released by the sample compound molecules. In this study, antioxidant activity tests were carried out on crude extracts, ethanol fractions, ethyl acetate fractions, n-hexane fractions, and isolates, using positive controls or ascorbic acid comparators. The results of antioxidant activity tests are shown in Table 7.

The IC_{50} value was inversely correlated with the

antioxidant activity of a sample, indicating that a lower IC_{50} value signifies a higher antioxidant potential. Generally, a compound is classified as exhibiting extreme antioxidant activity if its IC_{50} value is less than 50 ppm, strong activity if between 50-100 ppm, moderate activity if between 100-150 ppm, weak activity if between 150-200 ppm, and very weak activity if it is greater than 200 ppm. The results of the antioxidant activity assay across various sample concentrations (5, 20, 40, 60, and 80 ppm) demonstrated a corresponding increase in the antioxidant efficacy. The relationship between concentration and antioxidant activity is clearly depicted by the linear regression curve. A coefficient of determination (R^2) value close to or equal to 1 signifies excellent data fit for the analysis.

The IC_{50} values for ethanol, ethyl acetate, and n-hexane fraction were found to be 26.51 ppm, 286.82 ppm, and 278.12 ppm, respectively. The ethyl acetate and n-hexane fractions exhibited significantly high IC_{50} values, indicating weak antioxidant activity, likely because of the limited presence of compounds with antioxidant properties in these fractions. In contrast, the ethanol fraction, with an IC_{50} value of 26.51 ppm, exhibited extreme antioxidant activity. This enhanced activity was attributed to the presence of numerous bioactive compounds in the ethanol fraction, which showed positive results in phytochemical screening for flavonoids, tannins, and steroids.

Table 7. Antioxidant activities against DPPH

Samples	Consentrations (ppm)	Inhibition (%)	Regression	IC_{50} (ppm)	Activity
CE	5	37.27	y=0.7826x+34.803 R^2=0.9896	19.41	***
	20	49.90			
	40	68.59			
	60	83.85			
	80	94.82			
EF	5	29.76	y=0.8267x+28,084 R^2=0,9896	26.51	***
	20	45.05			
	40	64.34			
	60	79.19			
	80	91.53			
EaF	5	41.05	y=0.0328x+40.952 R^2=0.9043	286.82	*
	20	41.98			
	40	41.76			
	60	43.02			
	80	43.65			
HeF	5	17.26	y=0.1212x+16.18 R^2=0.9739	278.12	*
	20	17.70			
	40	21.62			
	60	23.26			
	80	25.97			
Ascorbic Acids	1	24.72	y=2.7261x+34.496 R^2=0.8152	5.68	***
	5	57.08			
	10	72.04			
	15	75.44			
	20	82.21			
IE	5	31.78	Y=0,6802x+24.224 R^2=0.9435	37.89	***
	20	38.22			
	40	44.12			
	60	62.83			
	80	83.58			

(***Very strong antioxidant activity; *Low activity).

IE has an IC_{50} value of 37.89 ppm, which is relatively higher than **CE** IC_{50} value (19.41 ppm, which is allegedly due to the possibility of a synergistic effect, wherein the **CE** extract contains many secondary metabolite compounds. At the same time, only one compound was identified in the isolate. The synergistic effect of active ingredients is a condition in which the effect produced by the active compounds is greater than the sum of the individual effects of each active compound [29]. These findings possess similar activity;

previous studies reported leaves extract *H. littoralis* exhibit significant antioxidant activity with the IC_{50} 26.30 ppm [35]. In addition, bark extracts of Heritiera fomes showed potent antioxidant activity with an IC_{50} value of 22 ppm [36].

3.4. Anti-inflammatory activities

This anti-inflammatory study was conducted using the rat hind paw edema method or the formation of artificial inflammation on the soles of the hind feet of male white rats using carrageenan as an inducer, and the profile of the increased volume of rat hind paw edema [37]. Carrageenan is an inflammatory inducer that provides a more sensitive response to anti-inflammatory drugs than other irritant compounds. Carrageenan-induced edema lasted 6 h and gradually decreased within 24 h. Edema occurs because of the release of inflammatory mediators such as histamine, bradykinin, and prostaglandins [38]. Anti-inflammatory activity tests were carried out on the fractions and isolates of *Heritiera littoralis.* The test animals were male mice aged 2-3 months weighing 20-30 g. Before testing, the mice were not fed for 18 h, so that there was no influence of other substances on the test results. After being injected with 1% 0.1 ml of carrageenan, the mice were left for 30 min to induce inflammation occurred in the left leg of the mice.

3.5. The Effect of Extract and Isolate in Changing Edema Volume

Data obtained from measuring the volume of edema in the paws of mice every hour in all groups produced results, as shown in Table 8.

Table 8. Data on Measurement of Edema in Mice

No.	Groups	Doses (mg/KgBW)	Volume of Edema (mL) During Observation (min)							
			V_o	V_t	60	120	180	240	300	360
1.	C-	10	0.19	0.29	0,33	0.34	0.36	0.37	0.38	0.38
2.	C-	10	0.18	0.30	0,31	0,33	0.35	0.35	0.36	0.36
3.	C-	10	0.19	0.31	0,30	0,32	0.34	0.36	0.37	0.37
	Averages ± SD		**0.19± 0.005**	**0.30± 0.010**	**0.31± 0.015**	**0.33± 0.010**	**0.35± 0.010**	**0.36± 0.010**	**0.37± 0.010**	**0.37± 0.010**
1.	C+	10	0.16	0.21	0.22	0.20	0.18	0.17	0.17	0.17
2.	C+	10	0.15	0.20	0.21	0,19	0.18	0.17	0.16	0.16
3.	C+	10	0.18	0.22	0.23	0,22	0.20	0.19	0.19	0.19
	Averages ± SD		**0.16± 0.015**	**0.21± 0.010**	**0.22± 0.010**	**0.20± 0.015**	**0.19± 0.011**	**0.18± 0.011**	**0.17± 0.015**	**0.17± 0.015**
1.	EF	15	0.14	0.20	0.21	0.19	0.18	0.17	0.17	0.16
2.	EF	15	0.13	0.19	0.19	0.20	0.17	0.17	0.16	0.16
3.	EF	15	0.13	0.18	0.19	0.18	0.18	0.16	0.15	0.15
	Averages ± SD		**0.13± 0.005**	**0.19± 0.010**	**0.20± 0.011**	**0.19± 0.010**	**0.17± 0.005**	**0.16± 0.005**	**0.15± 0.010**	**0.15± 0.005**
1.	IE	5	0.16	0.25	0.27	0.26	0.25	0.24	0.24	0.23
2.	IE	5	0.15	0.26	0.25	0.25	0.23	0.23	0.23	0.22
3.	IE	5	0.16	0.23	0.25	0.25	0.23	0.21	0.21	0.20
	Averages ± SD		**0.16± 0.005**	**0.24± 0.015**	**0.26± 0.011**	**0.25± 0.005**	**0.23± 0.011**	**0.22± 0.015**	**0.22± 0.015**	**0.21± 0.015**
1.	IE	10	0.16	0.25	0.25	0.23	0.22	0.21	0.21	0.20
2.	IE	10	0.15	0.22	0.24	0.24	0.21	0.20	0.18	0.18
3.	IEb	10	0.14	0.21	0.21	0.21	0.22	0.19	0.18	0.17
	Averages ± SD		**0.15± 0.010**	**0.22± 0.020**	**0.24± 0.020**	**0.23± 0.015**	**0.21± 0.005**	**0.20± 0.010**	**0.19± 0.017**	**0.18± 0.015**

Table 8 shows data on the decrease in edema in the feet of mice that measuring in every hour. The feet of mice marked using an ink marker are measured using a simple tool. Vo is the initial volume of the feet of mice without treatment. The feet of mice were given 1% carrageenan induction intraplantar and waited for 30 min, then measured on the tool to obtain the Vt value. Furthermore, the mice were given treatment in the form of 1% Na CMC solution, Na Diclofenac 10 mg / KgBW, ethanol fraction 15 mg / KgBW, and Isolate with doses of 5 mg / KgBW and 10 mg / KgBW orally. The volume of edema reduction in the feet of mice was recorded every 6 hr to obtain V1-V6. Based on the study's results, it was obtained that the **C-** showed a significant increase in edema volume. In the C-, Na-CMC inflammation continued to increase. It lasted for the 1st to 6th hr as Na-CMC is only a solvent for the drug media, so there is no stimulus in the form of drugs that can reduce edema. It can be seen in the table that the average edema in the **C-**

has the highest percentage, which is 87.84% compared to other treatment groups.

The ethanol fraction at a dose of 15 mg/KgBW, **EF,** showed a decrease from the first to the sixth hour. With this treatment, the average result of the decrease in edema volume was 30.88%. Moreover, the isolate with a dose of 5 mg/kg, **IEa** showed a decrease in edema volume from the first hour to the 6th hr, with an average decrease in edema volume of 29.23% was obtained. In comparison, in the 10 mg/KgBW isolate, there was an average decrease in volume of 39.79%. From the percentage of decrease in edema volume, it can be seen that the dose of catechin, with a dose of 10 mg/KgBW, and **EF** with a dose of 15 mg/KgBW showed a more significant decrease in edema compared to **IEa** at a dose of 5 mg/KgBW. These results suggest that the fraction produces anti-inflammatory activity and isolates *H. littoralis* seeds. It is known that *H. littoralis* seeds contain tannin compounds that have analgesic and anti-inflammatory activities, and tannins can inhibit the cyclooxygenase enzyme from prostaglandins, which has an anti-inflammatory analgesic effect [39]. Tannin compounds effectively inhibit COX-1 to reduce wriggling in mice [40]. In the comparison group, **C+** edema was reduced within the first hour of oral administration. This reduction persisted from the first to sixth hour. The percentage decrease in edema volume in the **C+** group was significantly more significant compared to the fraction and isolated test solution groups, with an average edema reduction of 15.78%. These findings indicated that sodium diclofenac exhibited a more pronounced anti-inflammatory effect than the test solutions. Sodium diclofenac was used as a positive control because of its potent anti-inflammatory properties and is frequently used as a standard in anti-inflammatory studies. Furthermore, **C+** is characterized by its rapid absorption in the body and relatively low side-effect profile compared to other anti-inflammatory drugs, such as piroxicam and indomethacin [41].

Table 9. Inflammation Percentage Results (%) in Various Times

Samples	Inflammation Percentage (%) during observation (min)					
	60	120	180	240	300	360
C- (Na CMC)	63.15	73.68	84.21	89.47	94.73	94.73
C+ (Na Diklofenac)	37.50	25.00	18.75	12.50	6.25	6.25
EF (Fraction 15 mg/KgBW)	53.84	46.15	30.76	23.07	15.38	16.66
IEa (Isolate 5 mg/KgBW)	62.50	56.25	43.75	37.50	37.50	31.25
IEb (Isolate 10 mg/KgBW)	60.00	53.33	40.00	33.33	26.66	20.00

The results of measuring the volume of mouse paw edema were the basis for calculating the average percentage of inflammation for each treatment. The negative control group exhibited no significant effect on suppressing carrageenan-induced inflammation, as no notable reduction in edema was observed up to the 360th min (Table 9). Conversely, all test groups, including the positive control group, fractions, and isolates, showed a gradual reduction in the percentage of inflammation over time. The mean percentage of inflammation for each group was subsequently used to calculate the percentage of inflammation inhibition and evaluate the extent of inflammation suppression achieved by each treatment (Table 10).

Table 10. % of Edema inhibition

No	Samples	Doses (mg/KgBW)	Edema Inhibition (%) during observation (min)						Averages
			60	120	180	240	300	360	
1.	C-	10	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2.	C-	10	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3.	C-	10	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4.	C+	10	49.10	68.33	86.02	93.40	93.75	93.75	80.72
5.	C+	10	44.61	68.00	78.82	85.88	93.34	93.34	77.33
6.	C+	10	56.89	69.84	86.80	93.79	94.14	94.14	82.60
7.	EF2	15	32.13	54.76	68.06	77.38	78.58	85.72	66.10
8.	EF2	15	36.09	35.38	67.42	67.42	76.93	76.93	60.02
9.	EF2	15	26.92	47.80	54.32	74.21	83.76	83.76	61.79
10.	IEa	5	6.69	20.82	37.12	47.21	50.00	56.25	36.34
11.	IEa	5	7.69	20.00	43.53	43.53	46.67	53.34	35.79
12.	IEa	5	10.92	23.65	48.04	65.07	67.01	73.60	48.04
13.	IEb	10	23.65	44.57	58.08	67.01	68.75	75.00	56.17
14.	IEb	10	16.92	27.99	57.64	64.70	80.00	80.00	54.53
15.	IEb	10	20.82	32.13	32.14	60.00	69.84	77.38	48.71

The anti-inflammatory effect is reflected in the average percentage of inflammation inhibition in each group. According to the data in the table, the **EF** at 15 mg/KgBW demonstrated a high inhibition percentage of 62.63%, while the **IEb** at 10 mg/kg showed an inhibition percentage of 53.13%. These values are comparable to the inhibition percentage of the positive control, which was 80.21%). The significant inhibition percentage of **C+** can be attributed to its pharmacological activity as an anti-inflammatory agent, primarily through the inhibition of the cyclooxygenase (COX) enzyme, which plays a critical role in synthesizing prostaglandins, which are key mediators of inflammation. Diclofenac sodium (**C+**) is classified as a non-selective NSAID-COX and reversible inhibitor. Additionally, NSAIDs are known to reduce vascular sensitivity to bradykinin and histamine, modulate T-lymphocyte activity, and mitigate the vasodilation caused by inflammation [42].

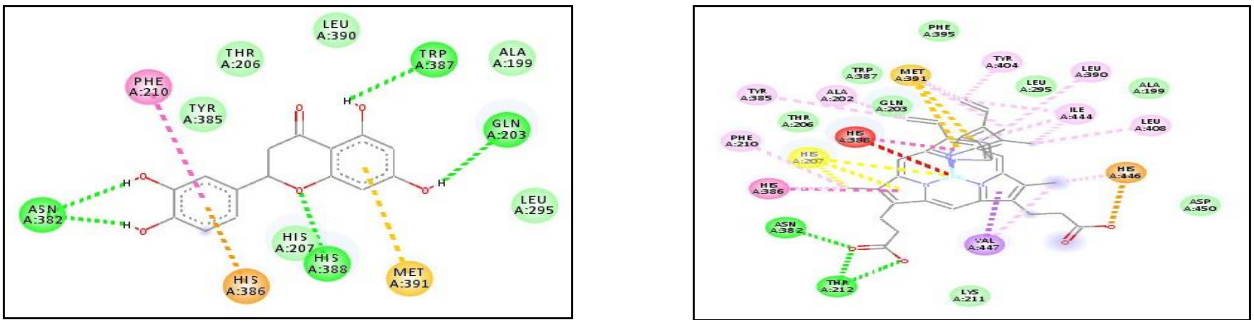
The results obtained from the edema inhibition percentages of various comparisons, including the ethanol fraction at a dose of 15 mg/kg BW and catechin isolates at doses of 5 mg/KgBW and 10 mg/KgBW, exhibited differences. These results were subsequently analyzed using a one-way ANOVA test conducted with SPSS 22 to assess any significant differences in anti-inflammatory activity among the treatment groups. In cases where the assumptions for one-way ANOVA were not satisfied, the analysis proceeded with the BNT (Smallest Real Difference) test, utilizing the post hoc LSD method to identify significant differences between treatment groups ($p>0.05$). Therefore, a post-hoc LSD test was performed to determine which groups exhibited statistically significant differences.

Table 11. Post LSD Test Results on Anti-Inflammatory Testing of Fractions and Isolates

Samples	Doses (mg/KgBW)	%Inhibition
C-	10	0.00[a]
C+	10	80.21[b]
EF	15	62.63[c]
IEa	5	40.05[c,d]
IEb	10	53.13[c]

[a,b,c]Superscripts with distinct letters within the same column are significantly different ($P<0.05$). SD represents the Standard Deviation.

Based on the Post LSD test in Table 11, all doses showed statistically significant differences in inhibition compared to the negative control. Meanwhile, it can be seen that there is no significant difference between the percentage inhibition of the 15 mg/KgBW fraction and the 10 mg/KgBW isolate, both doses have almost the same percentage of inflammation inhibition. When compared to the positive control, almost all doses showed significant differences, indicating that the doses in the extract and isolate have anti-inflammatory potential.



(c) (d)

Figure 5. The bioactive compounds interaction; (a) Catechin/COX-1; (b) Flavan 3,6,7,4',5'-pentaol/COX-1; (c)Eriodictyol/COX-1; (d) native ligand COX-1

in sillico assays were conducted on flavan-3,6,7,4',5'-pentaol, catechin, and eriodictyol through molecular docking using the PyRx program, the AutoDock Tools wizard with predefined grid box sizes and coordinates established during validation. The data revealed the binding energy values and interaction types, specifically hydrogen bonds. Binding energy represents a scoring function derived from the conformation of the ligand within the macromolecule at equilibrium. A negative binding energy indicates a state of equilibrium, where lower (more negative) values correlate with stronger ligand-receptor interactions and a lower inhibition constant. A compound was considered to exhibit strong inhibition when the inhibition constant was ≤100 μM, whereas values ≥100 μM indicated weak inhibition.

A lower binding energy signifies a more stable ligand-protein complex. Therefore, the conformation of the test compound with the lowest binding energy and specific interactions with amino acid residues at the binding site was selected. Docking results for flavan-3,6,7,4',5'-pentaol, catechin, and eriodictyol with the COX-1 receptor identified eriodictyol as the best-performing compound, with a free binding energy of -8.01 kcal/mol and an inhibition constant of 1.34 μM. Eriodictyol forms four hydrogen bonds with the amino acid residues ASN 382, TRP 387, GLN 203, and HIS 386. Hydrogen bonds

serve as key parameters for pharmacological interactions between drugs and receptors, and play a critical role in protein stability. The presence of these bonds in molecular dynamics simulations indicates a strong affinity between the compound and target receptor.

It was confirmed and reported by previous studies that among the compounds, eriodictyol exhibited the highest binding affinity with a free binding energy of -8.01 kcal/mol and an inhibition constant of 1.34 μM, indicating a strong and spontaneous interaction with the COX-1 active site. Key interactions include hydrogen bonds formed with amino acid residues ASN 382, TRP 387, GLN 203, and HIS 386, which are critical for stabilizing the ligand-receptor complex. These interactions suggested the significant potential of eriodictyol as a COX-1 inhibitor.

Catechin and flavan-3,6,7,4',5'-pentaol also showed substantial binding affinities, but their free binding energy values were less negative than that of eriodictyol, implying relatively weaker interactions. The docking results highlighted the role of hydrogen bonds and other non-covalent interactions in mediating the binding efficiency of these compounds. These findings align with existing studies that demonstrated the efficacy of polyphenolic compounds, such as flavonoids, in modulating COX enzymes, thereby reducing inflammation through the inhibition of prostaglandin synthesis.

5. Conclusion

Our study demonstrated that the isolate, characterized using UV-Vis spectroscopy, exhibited two absorption peaks at 218 nm and 280 nm, indicative of hydroxyl groups and electron transitions associated with the carbonyl group. FTIR analysis further confirmed functional groups with absorption bands at 3346.55 cm^{-1} (-OH group), 2925.67 cm^{-1} (C-H alkane), and 1521.35 cm^{-1} (C=C aromatic), supporting the identification of the isolate as catechin. Anti-inflammatory evaluation using the rat hind paw edema model revealed the highest inhibition percentage for sodium diclofenac (80.21%), followed by the ethanol extract at 15 mg/KgBW (62.63%) and the isolate at 10 mg/KgBW (53.13%). Additionally, antioxidant testing via the DPPH method indicated that the ethanol fraction possessed notable antioxidant activity, with an IC_{50} value of 26.51 ppm, compared to ascorbic acid as a positive control, which had an IC_{50} of 5.68 ppm. These findings highlight the potential of *H. littoralis* seeds as a source of bioactive compounds with potent anti-inflammatory and antioxidant activities.

Declarations

Author Contributions

Conceptualization, L. T., M. L.; methodology, S. E. L.; software, I.L.T., Y.; validation, I.L.T., M.L., and Y.; formal analysis, I.L.T.; investigation, S., E.L.; resources, I.L.T., M. I.; data curation, I.L.T., S., E.L.; writing—original draft preparation, I. L. T.; writing—review and editing, I.L.T., M.L.; visualization, S., E.L.; supervision, M.L.; project administration, I.L.T.; funding acquisition, I.L.T. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The animal study protocol was approved by the Institutional Review Board (or Ethics Committee) of the Faculty of Medicine and Health Sciences, Universitas Jambi. Ethical number: 2603/UN21.8/PT.01.04/2024

Informed Consent Statement

Not Applicable

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this manuscript.

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