

Anti-Inflammatory Activity and Phytochemical Profile from the Leaves of the Mangrove

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Anti-Inflammatory Activity and Phytochemical Profile from the Leaves of the Mangrove *Sonneratia caseolaris* (L.) Engl. for Future Drug Discovery

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Abstract

The increasing demand for effective and natural anti-inflammatory agents prompts an investigation into the properties of *Sonneratia caseolaris* (L.) Engl., a plant traditionally used in medicine. This study aimed to explore the ability of *S. caseolaris* leaves extract to inhibit inflammation and accelerate wound healing. *S. caseolaris* leaves were collected from Tanjung Api-Api area, Banyuasin, South Sumatra. The method involved carrageenan induction in rat paws as an inflammatory model. The results showed that the most effective dose was found in the group with a dose of 150 mg/kg BW measured using a digital caliper and plethysmometer. The qualitative phytochemical test contain alkaloids, steroids, flavonoids, and phenols. The results GC-MS analysis were thought to contain aldehydes, glucosinolates, coumarins, esters, terpenoids, alcohols, lipids, tocopherols, and steroids. Due to their diverse range of mechanisms of action, coumarins show significant promise in mitigating inflammation and hold potential for treating inflammatory conditions. This study provides new insights related to the potential of *S. caseolaris* as a source of natural anti-inflammatory agents, supporting public comprehension regarding the utilization of traditional herbal remedies.

Keywords

Anti-Inflammatory, *S. caseolaris*, Leaves, Phytochemical Profile, Drug Discovery

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1. INTRODUCTION

Inflammation is a natural reaction to harm and irritation resulting from infection, allergic reactions, or exposure to chemicals (MacLeod and Mansbridge, 2016). In inflammatory situations, common signs include vasodilation, which causes a rise in temperature and results in red discoloration. Increased vascular permeability leads to swelling, while nociceptors are physically and chemically stimulated, causing pain. Disruption of tissue structure results in the loss of normal tissue function (de Araújo et al., 2016; Bai et al., 2021). Frequently used clinical treatments for inflammatory conditions involve the use of anti-inflammatory compounds, both nonsteroidal and steroidal in nature (Shaikat et al., 2023). The use of steroid drugs as anti-inflammatory agents is currently controversial due to their many side effects (Itoh et al., 2023; Tsujimae et al., 2024). Therefore, it is necessary to be expanded new agents with stronger anti-inflammatory activity but with fewer side effects.

Nature is the ultimate source of all living things, and its various medicinal plants have been a resource for obtaining cures for various diseases since the dawn of human civilization

(Kundu et al., 2023). Despite the continuous improvement of modern techniques through methods like combinatorial chemistry or computational drug design, over fifty percent of modern pharmaceuticals originate from medicinal plants (Wainwright et al., 2022). Medicinal plants play a crucial role as a natural reservoir for combating enduring illnesses, offering a diverse array of treatments for various diseases (Ríos and Andújar, 2020; Maldonado Miranda, 2021). Native medicinal plants are a new source of anti-inflammatory agents with more tolerable side effects (Nascimento et al., 2021).

Mangroves are arboreal species found in tropical and subtropical coastal areas influenced by tidal fluctuations, primarily situated within the belt spanning from the northern to southern tropics (Biswas and Biswas, 2021; Anu et al., 2024). Several bioactive compounds with diverse effects, including insecticidal, antioxidant, and antimicrobial properties, have been extracted from mangrove plants (Roziwan et al., 2020, 2023b,a). Various biologically active compounds, including limonoids, alkaloids, glycosides, terpenoids, steroids, flavonoids, esters, phenols, lactones, aliphatic alcohols, amides, acids, aliphatic ketones, quinones, and benzodioxols, have been extracted from

48 different parts of mangrove trees, such as bark, stems, leaves, fruits, and seeds (Parthiban et al., 2023). The *Sonneratia* genus thrives continuously in coastal areas where land meets the sea, encountering difficulties like oxygen-deprived soil, increased salinity, and strong ultraviolet radiation throughout the year (Nasrin et al., 2021). *Sonneratia* adapted to its environment and metabolism in unique ways to deal with these problems. For example, it developed high osmotic pressure, adaptive root growth, and salt secretion mechanisms. These adaptation systems allow *Sonneratia* to survive harsh environmental conditions and produce unusual secondary metabolites (Liu et al., 2023). In addition, *Sonneratia caseolaris* is also one of the mangrove plant species known to have a variety of pharmacological activities.

Previous studies have shown that mangrove leaves contain various bioactive compounds. One of the compounds found was a triterpenoid compound, which has been demonstrated to possess properties that reduce inflammation (Kundu et al., 2022). In addition, flavonoid compounds are also found in mangrove leaves, which are also known to have strong antioxidant activity properties (Rozirwan et al., 2023a). A previous study indicated that the ethanol extract derived from *S. caseolaris* leaves contains a diverse range of phytochemicals, including phenolics, alkaloids, flavonoids, saponins, and tannins (Rahim and Abu Bakar, 2018). According to (Komakech et al., 2019), these compounds hold promise for inhibiting the function of proinflammatory enzymes, reduce the production of inflammatory mediators, and affect inflammatory signaling pathways.

45 However, the novelty of our work lies in a comprehensive exploration of the chemical compounds found in the leaves of *S. caseolaris*, aiming to identify previously undiscovered active components. This study merges ethnobotanical knowledge with modern scientific techniques, presenting a unique approach that is poised to contribute significantly to the advancement of novel, effective, and sustainable anti-inflammatory medicines. The exploration of novel compounds in *S. caseolaris* adds a distinctive dimension to the ongoing efforts in the field, promising innovative solutions to the challenges posed by inflammatory diseases.

2. EXPERIMENTAL SECTION

2.1 Materials

The materials and specifications used in this research are White Rats (*Rattus norvegicus*), Aquades, Seaweed Carrageenan, Carboxymethyl Cellulose Sodium (Shandong Yulong, China), Sodium Diclofenac (Pharos, Indonesia), Ethanol, HCL, and NaOH from Merck KGaA, Darmstadt, Germany. Mangrove leaf extract was obtained through the extraction process using a Biobase RE-301 Rotary Evaporator (China). Edema volume measurements were taken using the Orchid PLM01 Plethysmometer (Orchid Scientific, India) and a digital caliper with 6 carbon (Nankai, Japan Technology).

Identification, preparation, maceration, deconstruction, and antioxidant activity tests on samples were carried out at the Ma-

43 rine Bioecology Laboratory; phytochemical tests were carried out at the Agricultural Products Chemistry and Microbiology Laboratory; and screening of secondary metabolite compounds was carried out at the Integrated Testing Laboratory, Sriwijaya University. Mangrove identification refers to Giesen et al. (2007).

2.2 Method

2.2.1 Leaf Collection

This study was conducted in October 2023. The leaf samples used were *Sonneratia caseolaris* (L.) Engl. species obtained from the innermost zonation close to the sea with muddy areas lined up along the Tanjung Api-Api port in Banyuasin Regency, South Sumatra (Figure 1). Mangrove forests in the area are brackish forest ecosystems that grow in the area between land and sea waters (Rozirwan et al., 2023c). Mangroves also provide a unique and productive habitat for various types of flora and fauna, including fish, shrimp, crabs, birds, and other animals (Rozirwan et al., 2022).

2.2.2 Plant Maseration and Extraction

S. caseolaris mangrove leaves as much as 200 gr (dry weight) that have been mashed and macerated with ethanol solvent as much as 1 L (1: 5 b/v) for 2 x 24 hours. According to Rozirwan et al. (2023c), ethanol solvents that are polar are more effective for extracting secondary metabolites. The result of maceration in the form of a solution is then filtered with a filter. The macerated mixture was subjected to evaporation using a water bath set at 40°C until the solvent evaporated entirely, resulting in the formation of a paste-like substance (crude extract) from mangrove leaves. This crude extract was subsequently kept at ambient temperature.

2.2.3 Preparation of 1% Carrageenan Solution

41 The carrageenan solution was formulated by weighing 0.1 g of carrageenan, then dissolved with 10 mL of physiological saline (NaCl 0.9%), so that a 1% carrageenan solution was obtained (Fehrenbacher et al., 2012; Elfaruk et al., 2021).

2.2.4 Preparation of a Suspension of Na. CMC 0.5%

The preparation of 0.5% Na.CMC suspension refers to Liang et al. (2023), a total of 1.5 g Na. CMC is sprinkled evenly into a mortar that already contains 100 mL of heated distilled water, stirred until a clear gel is achieved, diluted with a little water, put into a 100-mL volumetric flask until a transparent mass is formed, and then added with distilled water until the mark line is reached.

2.2.5 Diclofenac Sodium Suspension Preparation

A total of 10 diclofenac sodium tablets (each tablet contains 50 mg diclofenac sodium) were weighed and then crushed. Diclofenac sodium tablet powder was weighed according to the dose calculation, then put into a 5 mL volumetric flask and suspended in 0.5% Na-CMC, and enough volume was added to make it 5 mL (Das et al., 2023).

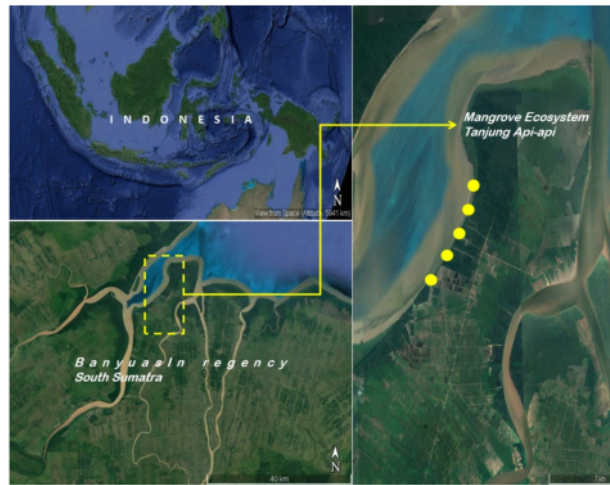


Figure 1. Map of Mangrove Leaves Collection

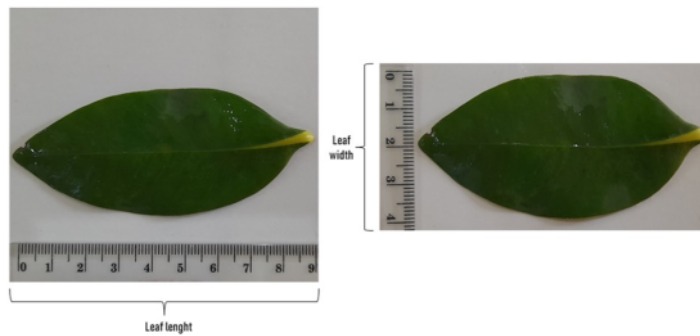


Figure 2. Leaf of *Sonneratia caseolaris* (L.) Engl.

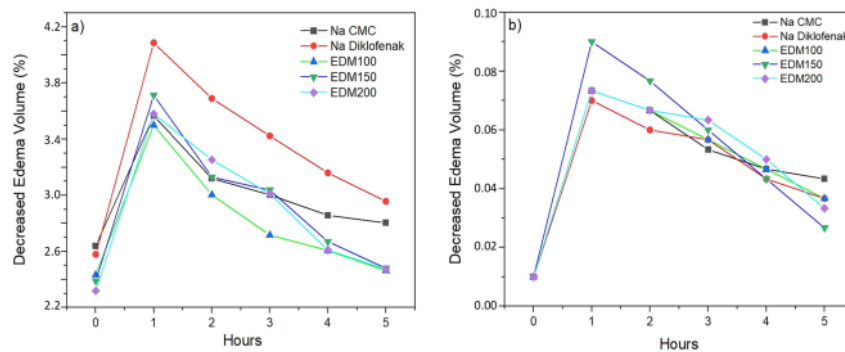


Figure 3. Reducing the Volume of Edema on the Soles of Rats' Feet. a) Plethysmometer, b) Digital Vernier Caliper

2.2.6 Preparation of *S. caseolaris* Leaves Extract Suspension Doses of 100 mg/kg BW, 150 mg/kg BW, and 200 mg/kg BW

Ethanol leaf extracts were prepared in suspension form with three different doses: 100 mg/kg BW, 150 mg/kg BW, and

200 mg/kg BW. Portions of 100 mg, 150 mg, and 200 mg of ethanol extract from *S. caseolaris* leaves were placed into a mortar. Then, 0.5% Na-CMC suspension was gradually added

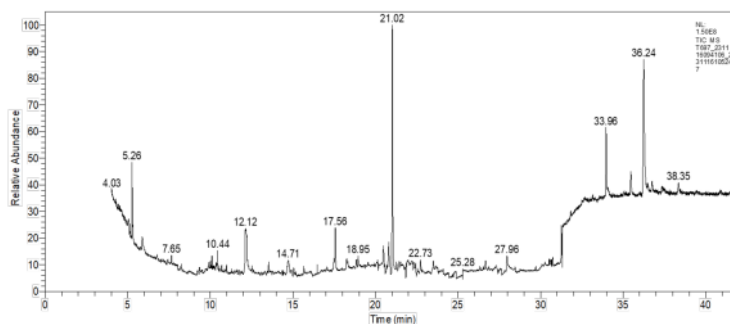


Figure 4. Analysis Chromatogram of Mangrove Leaves Extract *S. caseolaris*

while crushing until a uniform mixture was obtained. Finally, the mixture was transferred into a 10 mL volumetric flask and diluted to the mark line with 0.5% Na-CMC suspension.

2.2.7 Anti-Inflammatory Activity Assay

In this study, the carrageenan induction method on the soles of rat feet was used as an experimental method which was modified, referring to (Farida et al., 2022). Test animals included as many as 15 rats, which were divided into 5 experimental groups. The experiment was repeated three times, using three rats in each repetition. During the testing period, rats were weighed and randomly assigned to different groups, including a negative control group, a positive control group, and a test group. Each test group consisted of three rats.

Before treatment, each rat was given a boundary mark on the hind paw joint so that when measuring using a plethysmometer, the position was always the same. As a first step before treatment, the volume of the rat's paw was measured to determine the initial volume (V_0). Measurements were made using two measurement tools, namely a plethysmometer and a vernier caliper, with the aim of obtaining more accurate and comprehensive results. Combination: The use of these two tools allows for more complete measurements, especially if the object being measured involves changes in volume and length dimensions simultaneously.

Test animals were induced with 1% carrageenan in as much as 0.1 mL. The mice were then treated orally using a sonde at the third hour after the induction. The edema volume of the mice's feet was measured after treatment every 30 minutes for 5 hours. The volume of edema was determined based on the increase in the plethysmometer and Vernier caliper. Mice in each group were administered treatment according to the following procedure:

- The positive control group received diclofenac sodium at a dosage of 0.13 mg/kg BW.
- The group (negative control) was given Na. CMC 0.5% 0.5 mL (500 mg)/100 g BW.
- Test group 1 was given 100 mg/kg BW of *S. caseolaris* leaves ethanol extract.
- Test group 2 was given *S. caseolaris* leaves ethanol extract

150 mg/kg BW.

- Test group 3 was given 200 mg/kg BW of the ethanol extract of *S. caseolaris* leaves.

2.2.8 Qualitative Phytochemical Analysis

Qualitative phytochemical tests of *S. caseolaris* leaves extracts were carried out by qualitative methods as described in Harborne (1998), Bouabida and Dris (2022), and Olasunkanmi et al. (2022) in the form of alkaloid, flavonoid, terpenoid/steroid, saponin, and tannin tests.

2.2.9 Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

An analysis was performed to identify the bioactive compound constituents present in the *S. caseolaris* leaf extract. The spectral graph from the analysis findings was cross-referenced with the data repository in Wiley Library 7 (Hossain et al., 2017; Rahim and Abu Bakar, 2018).

2.3 Data Analysis

The observed variable is the change in the volume of inflammation in the sole of the test animal's foot per unit of time that has been determined. The use of the formula (1) for percent inflammation refers to Su et al. (2011).

$$\text{Percent Inflammation} = \left(\frac{V_t - V_o}{V_o} \right) \times 100\% \quad (1)$$

Where V_o is the initial volume of the mouse paw and V_t represents the inflammation of the mouse paw after treatment.

2.4 Statistical Analysis

Data analysis with the one-way ANOVA test is used to see differences in the mean of data groups with more than two treatments (Rozirwan et al., 2023d; García-Suárez et al., 2021). If the results obtained are significantly different or show that the p -value < 0.05 is considered significant, subsequently, the Tukey Honestly Significant Difference (HSD) test was employed to ascertain any disparities in the mean of each treatment using the IBM SPSS v.26 program.

3. RESULTS AND DISCUSSIONS

3.1 Description of Mangrove Leaf

Leaves are the characteristic part of a mangrove species. In the process of identifying each mangrove species, morphological observation of leaf shape is very necessary to be able to determine the characteristics of *Sonneratia caseolaris* leaf shape (Figure 2). *S. caseolaris* has skinned leaves that are round in shape; the base of the yellow peduncle has no glands; and the leaf tip is rounded. The base of the leaf is narrowed, with a leaf width of 3–4 cm and a leaf length of 7–9 cm. ³¹

Various mangrove species are found along the Tanjung Api-api Port area, South Sumatra. The Tanjung Api-api area is an area affected by anthropogenic activities like agriculture, residential areas, and port operations can lead to alterations in the marine ecosystem (Rozirwan et al., 2024; Fitria et al., 2023). Mangroves can act as natural filters that help cleanse water of polluting substances such as heavy metals and excess nutrients (Rozirwan et al., 2020; Ivorra et al., 2021). It can protect the marine environment from pollution that may come from plantation and settlement activities. The *Sonneratia* genus is one of the mangroves that are in the leading and dominant zone around the area (Rozirwan et al., 2023d). *S. caseolaris* is a characteristic mangrove species that reproduces via non-viviparous means and serves as an essential element of mangrove ecosystems in the Indo-West Pacific area (Chen et al., 2008). According to Ragavan et al. (2014), the morphology of *S. caseolaris* leaves ranges from lanceolate to broadly elliptical, featuring either a pointed or rounded apex; its branches do not hang downwards; it blooms with 1–3 flowers; mature buds are elliptical and slender in the middle; and its fruits are relatively larger in size.

3.2 Characteristics of Mangrove Leaves Extract

Percentage weight shrinkage of wet and dry samples of *Sonneratia caseolaris* leaves are summarized in Table 1. Shrinkage percentage of water content of *S. caseolaris* leaves have a shrinkage percentage of 69% and a sample weight of 30.9%. The moisture content serves as a measure of the water content within the leaves. Excessive moisture levels can diminish leaf quality as they promote microbial growth (Zheng et al., 2011; Ramses et al., 2020). According to Brendel (2021), The water content decreases as the plant ages. The solvent used in maceration is 70% ethanol, with the aim of attracting both polar and non-polar compounds (Batubara et al., 2020). Ethanol is an amphipathic solvent that can dissolve compounds that are both polar and nonpolar (Hikmawanti et al., 2021). Mangroves often contain various types of compounds with polar and nonpolar properties, so ethanol can effectively extract a large number of diverse bioactive compounds (Altemimi et al., 2017; Acquaviva et al., 2023).

3.3 Anti-Inflammatory Activity

The graph of the lower edema volume shows that the 150 mg/kg BW dose of mangrove leaves extract works faster than the 100 mg/kg BW dose, the 200 mg/kg BW dose, and the

positive control (Figure 3). This is indicated by the decrease in the volume of edema of mangrove leaf extract dose 150 mg/kg BW continuously from 1 to 5 hours compared to other controls using both digital vernier caliper and plethysmometer. Based on Figure 3, the greatest anti-inflammatory effectiveness was obtained at a dose of 150 mg/kg BW leaf extract group using a digital vernier caliper and plethysmometer, which is effective for binding to receptors. The anti-inflammatory effect is evidenced by the lowest percentage of inflammation observed over the course of 5 hours.

Sonneratia caseolaris mangrove leaf extract has anti-inflammatory activity against carrageenan-induced test animals. According to Machle (2009), The strength of drug effects corresponds to the receptors they interact with or bind to. Maximum effect occurs when all receptors are engaged by the drug, so increasing the dosage does not enhance the anti-inflammatory effect. The carrageenan test is commonly employed as a model for acute inflammation in experimental animals and exhibits high sensitivity to nonsteroidal anti-inflammatory drugs (Bouyahya et al., 2022; Chitsaz et al., 2023). There is a complicated process by which carrageenan causes an inflammatory response. It does this by releasing several acute inflammatory mediators and making blood vessels more permeable (Annamalai and Thangam, 2017). Previous studies have documented the anti-inflammatory properties of various mangrove species. The genus *Rhizophora* exhibits a range of pharmacological effects, such as antifungal, antibacterial, antiseptic, anti-inflammatory, and wound-healing activities (Sormin et al., 2021; Dat et al., 2022).

3.4 Analysis of Variance (ANOVA) and Honestly Significant Difference (HSD)

The results of the ANOVA test of the treatment group of anti-inflammatory activity on the plethysmometer with the values of F count and F table, namely $5.09 > 2.71$ and on the term thrust $7.21 > 2.71$, declare the presence of a notable distinction and proceed with the Tukey Honestly Significant Difference (HSD) examination, as outlined in Table 2. The results of the Tukey HSD test state that each treatment group has a significant difference, which is indicated by different numbers in each treatment at each hour.

Based on Table 2, in the plethysmometer measurement method for the Na CMC treatment group, the letter notation (kl) is shown for 1 hour, (ij) 2 hours, (ef) 3 hours, (d) 4 hours, and (c) 5 hours, which means that each treatment experienced a significantly different decrease in edema volume. Furthermore, Na Diclofenac is shown with the letter notation (jk) for 1 hour, (gh) 2 hours, (fg) 3 hours, (c) 4 hours, and (b) 5 hours, which is interpreted as each treatment experiencing a significantly different decrease in edema volume. Then the test group received a 100 mg/kg BW dose of *Sonneratia caseolaris* leaf extract is indicated by the notation (kl) for 1 hour, (ij) 2 hours, (fg) 3 hours, (d) 4 hours, and (b) 5 hours, then the 150 mg/kg BW dose test group is indicated by the notation (m), which is the notation with the highest letter, which is interpreted as

Table 1. Percentage Shrinkage of Leaf Extract

Leaf Sample	Sample Weight (gr)		Percentage Depreciation (%)	Percentage Sample Weight (%)
	Wet	Dry		
<i>S. caseolaris</i>	1780	551	69	30,9

Table 2. Tukey HSD Further Tested the Results of *S. caseolaris* Leaves Anti-Inflammation

Methods Measurements	Group Treatment	Time (Hour)				
		1	2	3	4	5
Plethysmometer	Na CMC	0.073±0.006kl	0.067±0.006ij	0.053±0.006ef	0.047±0.006d	0.043±0.006c
	Na Diklofenak	0.070±0.10jk	0.060±0.010gh	0.057±0.012fg	0.043±0.006c	0.037±0.006b
	EDM100	0.073±0.006kl	0.067±0.006ij	0.057±0.006fg	0.047±0.006d	0.037±0.006b
	EDM150	0.090±0.010m	0.077±0.012l	0.060±0.000gh	0.043±0.006c	0.027±0.006a
	EDM200	0.073±0.006kl	0.067±0.006ij	0.063±0.006hi	0.050±0.000d	0.033±0.006b
Vernier caliper	Na CMC	3.57±0.25bc	3.12±0.09bc	3.00±0.13bc	2.86±0.09bc	2.80±0.10b
	Na Diklofenak	4.09±0.32c	3.69±0.31c	3.42±0.40bc	3.16±0.29bc	2.96±0.25ab
	EDM100	3.50±0.37c	3.00±0.16b	2.72±0.30ab	2.61±0.23ab	2.46±0.15ab
	EDM150	3.71±0.04bc	3.13±0.03ab	3.04±0.05ab	2.67±0.13ab	2.48±0.12a
	EDM200	3.58±0.24bc	3.25±0.48bc	3.01±0.47ab	2.61±0.21ab	2.47±0.03ab

Table 3. Qualitative Phytochemicals

Leaf Sample	Compound				
	Alkaloids	Steroids	Flavonoids	Saponins	Phenols
<i>S. caseolaris</i>	+	+	+	-	+
	+	+	+	-	+

the greatest inflammation of the edema at 1 hour, (l) 2 hours, (gh) 3 hours, (c) 4 hours, and (a) 5 hours, which is interpreted as each treatment experiencing a very significantly different edema volume decrease. Then the dose of 200 mg/kgBB is indicated by the letter notation (kl) for 1 hour, (ij) for 2 hours, (hi) for 3 hours, (d) for 4 hours, and (b) for 5 hours, which means that each treatment has a significantly different edema volume.

Furthermore, in the method of measuring the term thrust for the Na CMC treatment group, the same letter notation (bc) is shown from 1 hour to 4 hours, which means that the treatment is not significantly different, and at the 5 hour, there is a decrease in edema volume with the notation (b), which is interpreted as significantly different. Next, with the Na Diclofenac treatment group, the letter notation (c) is shown for 1 hour to 2 hours, indicating that the treatment is not significantly different, then there is a decrease in edema volume with a change in notation to (bc) at 3 hours to 4 hours, and again a decrease at 5 hours with the notation (ab). In the *S. caseolaris* leaf extract test group for a dose of 100 mg/kgBB indicated by notation (c) at 1 hour and changed at 2 hours with notation (b), which showed a real difference. Furthermore, the decrease in edema volume from 3 hours to 5 hours with notation (c) means that the treatment is not significantly different. Then, at a dose of

150 mg/kgBB indicated by the notation (bc) at 1 hour, there is a real difference with the notation (c), which means that the treatment is not significantly different. The decrease in edema volume from 2 hours with notation (ab) until 3 hours means that the treatment is not significantly different, and only at 5 hours is there a decrease in edema volume with notation (a), which is interpreted as a significantly different treatment. Furthermore, the dose of 200 mg/kg BW is indicated by notation (bc) at 1 hour to 2 hours, which is interpreted as not significantly different, and there is a decrease in edema volume at 3 hours to 5 hours with notation (ab).

The outcomes from the Tukey examination revealed that across the board category of reduction in the volume of edema on the soles of the feet of rats, there were differences in each treatment group. The results of the decrease in edema volume with different letter notation signs in the Tukey HSD test showed that in the 150 mg/kg BW dose of mangrove leaves extract in the digital vernier caliper and plethysmometer method. The use of a digital plethysmometer will make the results or value of the decrease in edema more thorough. According to [Bucan et al. \(2022\)](#), measurements with digital calipers and plethysmometers are reliable and can be applied interchangeably. A plethysmometer is a precise and easy measurement method for decreasing edema volume ([Zhang et al., 2020](#)).

Table 4. Retention Time, Peak Area, Name of Compounds, Formula, and Compound Group

Ret. Time	Peak Area %	Name of Compounds	Formula	Compound Group
5.26	3.71	Nonanal	C ₉ H ₁₈ O	Aldehydes (Hao, 2019)
12.13	9.16	Desulphosinigrin	C ₁₀ H ₁₇ NO ₆ S	Glucosinolates (Youssef et al., 2023)
14.71	2.84	5,8-Epoxy-3H-2-benzopyran, 4,4a,5,8-tetrahydro-5,8-dimethyl-, (4aα,5α,8α)-	C ₉ H ₆ O ₂	Coumarin (Bansal et al., 2013)
17.57	3.40	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	Ester (Habtemariam, 2019)
20.48	4.61	1-Hexadecanol, 2-methyl-6,9,12,15-	C ₁₇ H ₃₆ O	Alcohol (Prasanseang et al., 2023)
20.80	3.88	Docosatetraenoic acid, methyl ester	C ₂₃ H ₃₈ O ₂	Ester (Yu et al., 2020)
21.02	17.26	Phytol	C ₂₀ H ₄₀ O	Terpenoids (Hao et al., 2015)
21.97	3.70	1-Heptatriacotanol	C ₃₇ H ₇₆ O	Alcohol (Zaidan et al., 2019)
22.18	3.07	7-Methyl-Z-tetradecen-1-ol acetate	C ₁₇ H ₃₂ O ₂	Ester (Oluwasina et al., 2023)
27.96	2.75	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C ₁₉ H ₃₈ O ₄	Ester (Syed et al., 2022)
31.34	9.64	1-Monolinoleoylglycerol trimethylsilyl ether	C ₁₈ H ₃₂ O ₂	Lipids (Taha et al., 2023)
33.96	6.72	α-Tocopheryl acetate	C ₃₁ H ₅₂ O ₃	Tocopherol (Zahid et al., 2019)
36.24	17.45	β-Sitosterol	C ₂₉ H ₅₀ O	Steroids (Petrova et al., 2023)

3.5 Qualitative Phytochemical Analysis of Leaves Extract

Qualitative phytochemical analysis of leaves extract were positive for alkaloids, steroids, flavonoids, and phenols (Table 3). Bioactive compounds contained in *Sonneratia caseolaris* leaves include alkaloids, steroids, and flavonoids. The qualitative phytochemical tests for *S. caseolaris* indicate the presence of bioactive compounds like flavonoids and phenols, which are believed to exhibit potent biological activity, such as anti-inflammatory effects. While saponins had negative results with no shown or detected foam at the time of testing.

Qualitative phytochemical tests on *S. caseolaris* leaves extracts were positive for bioactive compounds that lead to anti-inflammatory activity. In line with the results of the study Kalasuba et al. (2023), the mangrove species *Rhizophora stylosa* is rich in alkaloids, flavonoids, phenolic acids, tannins, terpenoids, saponins, and steroids and is widely used in traditional medicine for its anti-inflammatory, antibacterial, antioxidant, and antipyretic effects. Flavonoids and saponins are recognized for their anti-inflammatory properties (Meshram et al., 2016; Chang et al., 2023). According to Syahidah and Subekti (2019), various metabolite compounds like alkaloids, flavonoids, phenolics, tannins, and terpenoid saponins are present in mangrove plants. Consequently, this plant is extensively utilized in traditional medicine across diverse regions of Indonesia, notably for


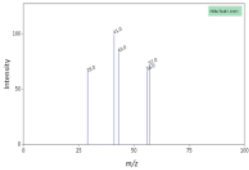
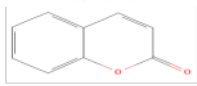
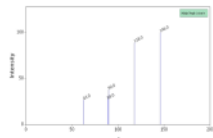
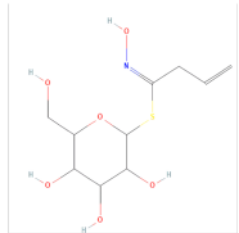
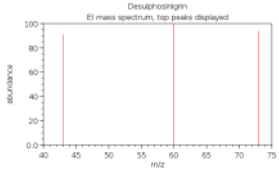

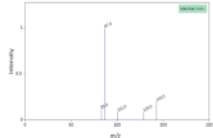

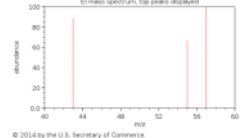
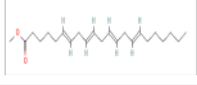
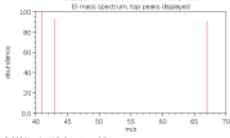
its anti-inflammatory and antipyretic properties (Arbiastutie et al., 2021; Al Kazman et al., 2022).

3.6 GC-MS Analysis of *S. caseolaris* Leaves Extract

The graph of GC analysis results obtained 13 peak points (Figure 4). Compounds identified based on chromatogram peak heights were matched identically to the library data base: WILEY 7 (Table 4). The chromatogram analysis of *Sonneratia caseolaris* leaf extract fractions clearly showed the presence of 13 secondary metabolite compounds, which were found in the library database (WILEY 7). The main groups of compounds that were found were aldehydes, glucosinolates, coumarins, esters, terpenoids, alcohols, lipids, tocopherols, and steroids. Information related to compound names, chemical structures, and mass spectra is summarized in Table 5.

According to the findings from Table 4, the majority of ethanol extracts from *S. caseolaris* leaves consist of aldehyde compound, glucosinolates, coumarins, esters, terpenoids, alcohols, lipids, tocopherols, and steroids. Nonanal, which is classified as an aldehyde compound, is a compound that is often found in various essential oils and can give a distinctive aroma to some plants. In addition, based on reports Tseng et al. (2023), aldehyde compounds are potent anti-inflammatory active compounds in *Cinnamomum cassia*. Desulphosinigrin, a type of glucosinolate, is also documented for its anti-cancer and antimi-

Table 5. Compound name, Chemical Structure, and Mass Spectral Information


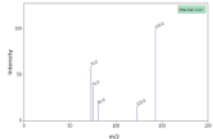

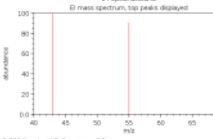
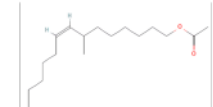
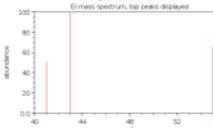
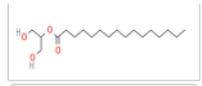
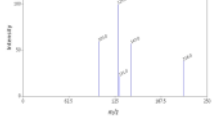
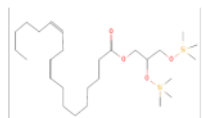
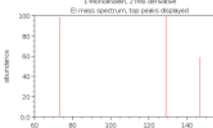
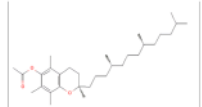
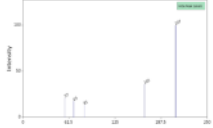
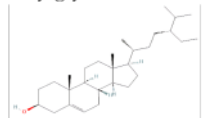
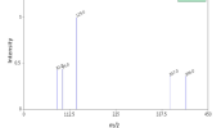
Compound Name, Chemical Structure, and Mass Spectra	
  Nonanal   5,8-Epoxy-3H-2-benzopyran, 4,4a,5,8-tetrahydro-5,8-dimethyl-, (4α,5α,8α)-	  Desulphosinigrin   Hexadecanoic acid, methyl ester
  1-Hexadecanol, 2-methyl-	  6,9,12,15-Docosatetraenoic acid, methyl ester

icrobial properties (Youssef et al., 2023). 4-methylsulfinylbutyl glucosinolate is a compound originating from the amino acid methionine, known for its antifungal, antioxidant, and antimicrobial properties (Elaiyaraja and Chandramohan, 2018).

Hexadecanoic acid, methyl ester, 6,9,12,15-Docosatetraenoic acid, methyl ester, 7-Methyl-Z-tetradecen-1-ol acetate, and Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, are classified as ester class compounds. Research results of Sokeng et al. (2020), it is evident that compounds within the ester group exhibit both acute and chronic anti-inflammatory

effects as well as central analgesic properties. 1-Hexadecanol, 2-methyl- and 1-Heptatriacotanol belong to alcohol compounds. So far, some alcohol compounds, especially those found in plant essential oils, can show anti-inflammatory activity that can help reduce inflammatory reactions in the body (Ali et al., 2015; Agarwal et al., 2022). Phytol is classified among terpenoid compounds, which represent one of the most diverse categories of natural substances, garnering significant interest because of their wide range of biological activities (Masyita et al., 2022). Terpenoids exhibit potent anti-inflammatory effects, making

Table 5. Cont.

Compound Name, Chemical Structure, and Mass Spectra	
  <p>Phytol</p>	  <p>1-Heptatriacontanol</p>
  <p>7-Methyl-Z-tetradecen-1-ol acetate</p>	  <p>2-hydroxy-1-(hydroxymethyl)ethyl ester</p>
  <p>1-Monolinoleoylglycerol trimethylsilyl ether</p>	  <p>α-Tocopheryl acetate</p>
  <p>β-Sitosterol</p>	

Source : (Kim et al., 2022).

them viable candidates for subsequent structural modifications and optimization (Ge et al., 2022; Ghallab et al., 2024). Another discovered component, 1-Monolinoleoylglycerol trimethylsilyl ether, has been reported as an antioxidant, antimicro-

bial, and anti-inflammatory agent (Mohan et al., 2015; Sezer and Uysal, 2021). α -Tocopheryl acetate is classified as a tocopherol compound. Based on the results of research Cassano (2012), each form of vitamin E (α -tocopherol) has biologi-

cal activity, with its natural configuration being 2R,4'R,8'R whose main function of vitamin E is as an antioxidant. The last compound is β -Sitosterol classified as a steroid compound. According to the results of research Chang et al. (2023), β -Sitosterol (BS) is present in a variety of plant seeds, vegetable oils, and nuts demonstrating diverse anti-inflammatory effects on peritoneal macrophages and peripheral tissues.

Furthermore, 5,8-Epoxy-3H-2-benzopyran, 4,4a,5,8-tetrahydro-5,8-dimethyl-, (4 α ,5 α ,8 α)- belongs to the class of coumarin compounds. Several naturally occurring compounds containing coumarinic groups have been documented to possess diverse biological activities (Menezes and Diederich, 2019; Sun et al., 2021). According to Annunziata et al. (2020), coumarin in the pharmaceutical field is known for its potential as an anticoagulant, which means it can affect blood clotting. Similar to isomeric flavonoids, coumarin is anticipated to impact the generation and elimination of reactive oxygen species (ROS) and affect processes associated with free radical-induced damage. Coumarin has the potential to alleviate tissue swelling and inflammation. Also, coumarins and their 7-hydroxy derivatives stop the production of prostaglandins, which encompasses intermediates of fatty acid hydroperoxides (Fylaktakidou et al., 2004). Several studies show that coumarin compounds have the ability to inhibit the enzymes cyclooxygenase (COX) and lipoxygenase (LOX), which are the main enzymes in the prostaglandin and leukotriene synthesis pathways (Lončarić et al., 2020; Chahal et al., 2023; Rudrapal et al., 2023). Through inhibiting the function of this enzyme, coumarin compounds can decrease the generation of inflammatory mediators, thus mitigating the inflammatory response (Al-Duhaidahawi et al., 2022; Chen et al., 2024). Coumarin compounds can also influence the activity of immune cells, which play a role in the inflammatory process (Sandhiutami et al., 2017; Rostom et al., 2022). This can be accomplished by diminishing the release of pro-inflammatory cytokines and hindering the migration of inflammatory cells to the inflammation site, or increasing the activity of immune cells responsible for the recovery and resolution of inflammation (Wu et al., 2022; Zhang et al., 2023; Chen et al., 2024).

Therefore, the ethanol extract derived from *Sonneratia caseolaris* leaves contains a diverse array of compounds that have been documented to possess anti-inflammatory, anti-cancer, antioxidant, and antimicrobial properties. Based on the multiple mechanisms of action involved, coumarins are promising compounds for reducing inflammation and may have potential for use in the treatment of inflammatory diseases. These findings open up great opportunities for the utilization of *S. caseolaris* leaves as a potential source for future traditional medicines, which can help people maintain their health.

4. CONCLUSIONS

S. caseolaris leaves extract has anti-inflammatory activity against carrageenan-induced test animals. The anti-inflammatory effect is denoted by the lowest percentage of inflammation observed during the 5-hour monitoring period, which is calcu-

lated as the average decrease. The largest dose of *S. caseolaris* leaves extract is found in the 150 mg/kg BW mangrove leaf extract group dose using a digital caliper and plethysmometer. *S. caseolaris* mangrove leaves positively contain secondary metabolites, namely flavonoids, steroids, alkaloids, and phenols. The findings from the GC-MS examination of *S. caseolaris* leaves extracts are thought to contain groups of aldehyde compounds, glucosinolates, coumarins, esters, terpenoids, alcohols, lipids, tocopherols, and steroids.

5. ACKNOWLEDGMENT

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