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Evaluation of the Potential Anti-inflammatory Activity of Sonneratia Alba Ethanolic Seed Extract in a Carrageenan-Induced Paw Edema Mouse Model

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Abstract

Sonneratia alba, locally known as "pagatpat," has been traditionally used in Filipino folk medicine to relieve inflammation, muscle pain, and sprains. This study explored the anti-inflammatory potential of the ethanolic seed extract of S. alba using a carrageenan-induced paw edema model in Swiss albino mice. Carrageenan was used to induce inflammation due to its ability to activate pro-inflammatory cytokines. The plant seed was collected, cleaned, air-dried, then powderized using blender (Ninja Foodi SS201 Power Blender and Processor), sifted using a metal mesh sieve no.120 to achieve the particle size 125µm and was macerated using 95% Ethanol in a 250 mL Erlenmeyer flask. Test groups received varying concentrations of the extract: (low, mid, and high) oral gavage, diclofenac sodium through oral route (positive control group), and NSS through oral administration (negative control group). Inflammation was induced through sub-plantar injection of carrageenan into the right hind paw, and paw swelling was measured using digital vernier caliper with the intervals (0, 1, 2, 3, 4, 5 hours) to assess edema progression. The 95% ethanolic seed extract of *S.alba* demonstrated a time-dependent anti-inflammatory effect in the acute paw edema model. Among the tested doses, the mid dose exhibited the greatest efficacy, producing the highest reduction in edema nearly comparable to Diclofenac sodium. The Post Hoc Tuckey HSD test showed a significant difference between the extract treated dose groups and the negative control ($P=0.0031$) starting at the second hour and continuing during the later observation periods, further supporting the anti-inflammatory potential of the extract. In one way ANOVA the anti-inflammatory activity of the seed extract becomes statistically significant at the second hour ($f = 17.45248$ and $p< 0.0001$) which continues to be significant in succeeding hours. Phytochemical analysis confirmed the presence of bioactive constituents such as alkaloids, terpenoids, flavonoids, phenols, steroids, saponins, and tannins, which may contribute to its anti-inflammatory activity. Acute toxicity testing showed that the extract was toxic at 5000mg/kg or higher, identified as the lethal dose. Overall, the findings support the potential of *S.alba* as a natural source of anti-inflammatory compounds and a possible alternative to synthetic nonsteroidal anti-inflammatory drugs (NSAIDs). These results provide a basis for future preclinical studies and possible pharmaceutical development of *S.alba* derived compounds while emphasizing the importance of proper dose evaluation.

Keywords: *Sonneratia alba*, Ethanolic Seed Extract, Anti-Inflammatory, Carrageenan, Induce Paw Edema

Chapter 1

1.1 Introduction

Inflammation is a vital biological response that helps maintain homeostasis and ensures tissue survival following injury, infection, or irritation, and is typically characterized by redness, swelling, heat, and pain. While essential for protection and healing, prolonged or chronic inflammation can hinder wound recovery and contribute to the development of various diseases. Conventional treatment options for inflammatory conditions often rely on nonsteroidal anti-inflammatory drugs (NSAIDs), such as diclofenac sodium, which, despite their efficacy, are frequently associated with adverse effects like gastrointestinal complications and hepatotoxicity (Casiple et al. 2025). An anti-inflammatory agent is a substance or drug that reduces, suppresses, or prevents the signs and processes of inflammation, including redness, swelling, pain, and heat, by inhibiting inflammatory mediators while maintaining the beneficial aspects of the immune response (Mahmoud et al. 2021).

Sonneratia alba is a mangrove tree in the family Lythraceae. *S. alba*, an arboreal mangrove species found along tropical and subtropical coasts, has been traditionally used by traditional healers, utilizing its pulp and extract as a poultice for treating muscular swellings and sprains. In Indian and Indonesian folkloric medicine, *S. alba* is commonly used for treating injuries such as wounds or sprains (Kulkarni and Manohar; Biswas and Biswas, 2021).

Members of the Lythraceae family were valued for their rich phytochemical profiles and in vivo anti-inflammatory potential. In experimental models, *Pemphis acidula*, a mangrove associate species, was found to contain flavonoids, tannins, saponins, and phenols, which were linked to measurable reductions in inflammatory responses in test animals (Sundari et al. 2022). The combined action of steroids, terpenoids, glycosides, polyphenols, amino acids, alkaloids, flavonoids, and tannins was responsible for the significant inhibition of carrageenan-induced paw edema in albino rats observed in an in vivo evaluation of ethanolic extracts from *Lagerstroemia speciosa* (pride of India) leaf, flower, and seed (Pavithran and Sujatha, 2022). These findings suggested that Lythraceae species, particularly the ethanolic seed extract of *S. alba*, may also contain phytochemical constituents that could serve as a natural alternative to synthetic anti-inflammatory agents. Despite existing literature on the applications and uses of *S. alba*, there were still limited studies on its seed as a potential anti-inflammatory agent.

This study aimed to address the existing gap by identifying an alternative natural source of anti-inflammatory drugs. It investigated the scientific literature regarding the anti-inflammatory potential of *S. alba* ethanolic seed extracts at low, mid, and high doses of LD_{50}. The anti-inflammatory activity was assessed via the carrageenan-induced paw edema model in mice, where inflammation was measured using a digital Vernier caliper. This provided a straightforward and precise method of assessing paw edema by directly measuring the thickness of the inflamed paw at specific time intervals (0, 1, 2, 3, 4, and 5 hours), allowing for accurate quantification of swelling (Zhang et al. 2022). The ethanolic seed extract was compared with the positive control group (diclofenac sodium) due to its rapid onset and consistent bioavailability, which ensures that edema inhibition occurs within a clear time window (1–5 hours) ideal for the carrageenan test. It is effective in managing pain and inflammation by inhibiting COX-2 (Dolanbay et al. 2021), thereby providing experimental evidence for its potential anti-inflammatory property.

1.2 Background of the study

Sonneratia alba is an arboreal species found in tropical and subtropical coastal areas influenced by tidal fluctuations and is primarily distributed within the belt spanning from the northern to southern tropics (Biswas and Biswas, 2021). Inflammation is a type of defense mechanism used by the body to prevent

pathogenic injurious agents from spreading further in the body (Kundu et al. 2022). It is triggered by numerous harmful stimuli, including pathogens and physical trauma, and is mediated by immunological responses (Rossi et al. 2021).

There are two main types of inflammation: acute and chronic. Acute inflammation, also known as short-term inflammation, lasts only for a few days, typically up to 4–6 weeks. It is characterized by pain, redness, swelling (formation of edema), heat, or loss of function. Acute inflammation can result from injury, infection, or exposure to substances such as bee stings or dust. In contrast, chronic inflammation is long-term inflammation that lasts for several months to years. The outcome of this study could support the development of naturally derived therapeutics for inflammatory conditions by providing safer and natural anti-inflammatory medications.

Anti-inflammatory drugs can interfere with the pathophysiology of inflammation, aiming to reduce tissue damage and improve patient comfort. Anti-inflammatory medications such as nonsteroidal anti-inflammatory drugs (NSAIDs) work by inhibiting the enzyme cyclooxygenase and are used to treat mild to moderate pain and control body temperature (Nunes et al. 2020). However, despite their therapeutic benefits, NSAIDs are associated with notable adverse effects, especially with long-term use. Common complications include gastrointestinal damage such as dyspepsia, ulcers, and bleeding, as well as cardiovascular and renal risks, including hypertension, myocardial infarction, and acute kidney injury (Cabassi et al. 2020).

These safety concerns had encouraged the exploration of natural anti-inflammatory agents as alternatives to synthetic drugs. Extracts from *Lagerstroemia speciosa* (banaba), a plant in the Lythraceae family, had demonstrated significant anti-inflammatory and antioxidant effects in experimental models, showing potential for alleviating inflammation-related conditions with a lower risk of gastrointestinal toxicity compared to NSAIDs (Pavithran & Sujatha 2022; Singh & Ezhilarasan 2022).

1.3 Conceptual Framework

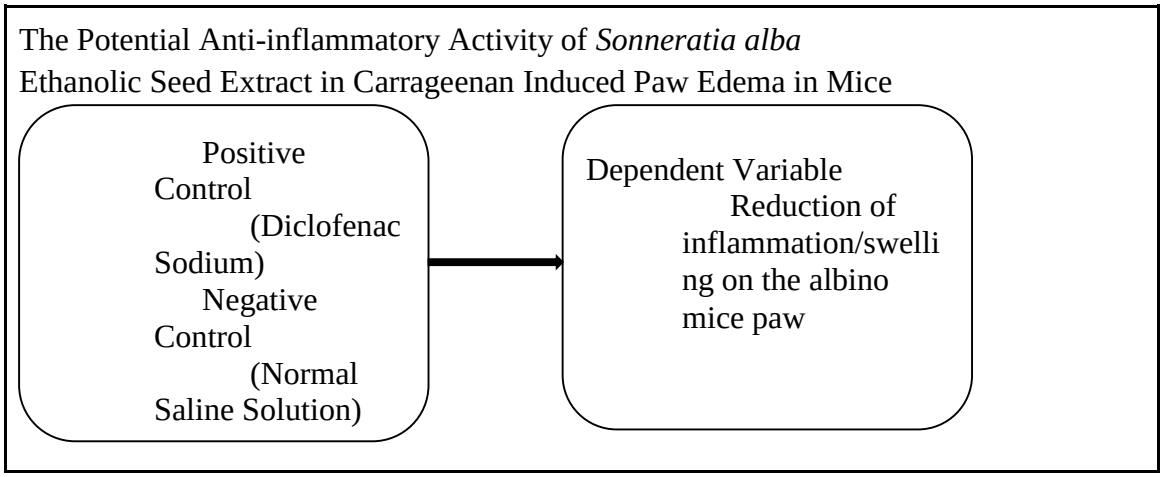


Figure 1. Conceptual Framework of the study

The figure above showed the conceptual framework on the effect of the independent variables, namely the positive control (diclofenac sodium), the negative control (normal saline solution), and the different concentrations of the plant extract (low, middle, and high dose of the LD_{50}) of *S. alba* on the dependent variable, which was the reduction of inflammation or swelling in the paw. Reduction of inflammation/swelling as the dependent variable was the key indicator of the anti-inflammatory process,

reflecting the decrease in inflammation size over time as a result of tissue regeneration and repair. This represented how different treatments were expected to influence the inflammatory response in the test subjects. This model aimed to determine the effectiveness of *S. alba* in treating inflammation compared to the positive control.

1.4 Research Objectives

The study determined the phytochemical composition, toxicity profile, and anti-inflammatory potential of the ethanolic seed extract of *Sonneratia alba* through percentage yield analysis, acute oral toxicity testing, and evaluation of its dose-dependent anti-inflammatory activity using the carrageenan-induced paw edema model.

1. To know the percentage yield of the ethanolic seed extract of *Sonneratia alba* via triplicate analysis of dried 10-gram plant sample.
2. To determine the phytochemical profile of the ethanolic seed extract of *S. alba.*
3. To evaluate the median lethal dose and the minimum toxic dose of the ethanolic seed extract of *S. alba* via 423 Acute Toxic dose test oral.
4. To assess the anti-inflammatory potential of the different concentrations of *S. alba* ethanolic seed extract using (using low, mid, and high dose of the LD50) using carrageenan induced paw edema.
5. To analyze if there is a significant difference between the ethanolic seed extract of *S. alba* and the positive control (Diclofenac sodium) in terms of its anti-inflammatory activity using carrageenan induced paw edema method.
6. To see if there is a significant difference among the different concentrations of ethanolic seed extract of *S. alba* namely: low, mid and high dose of median lethal dose.
7. To evaluate if the anti-inflammatory potential of *S. alba* ethanolic seed extract is concentration dependent (low, mid and high of median lethal dose).

1.5 Research Hypothesis

H0: There was no significant anti-inflammatory activity in carrageenan-induced paw edema in mice compared to the positive control (diclofenac sodium).

H1: There was a significant anti-inflammatory activity in carrageenan-induced paw edema in mice compared to the positive control (diclofenac sodium).

1.6 Significance of the study

This study aimed to assess the potential anti-inflammatory activity of *S. alba* seed extract and may provide significance to the following:

To the pharmaceutical industry: The findings could introduce a natural compound with potent anti-inflammatory properties. This may result in the development of new, safer pharmaceutical formulations, reducing adverse effects associated with synthetic drugs like NSAIDs.

To Clinical practice: The results may provide healthcare professionals with a safe and effective plant-derived alternative for managing inflammatory conditions.

To the Community: The study highlighted the medicinal value of a readily available local resource, *S. alba*. It may encourage the sustainable use of plants and contribute to community self-reliance by offering a natural alternative to expensive synthetic drugs.

Academia: The research served as a reliable reference for healthcare professionals, the pharmacy department, and future researchers interested in plant-based materials for inflammation.

To Future researchers: This research may serve as a valuable reference for comparison with other plants or extraction methods and may help in further studies on *S. alba* or other plants with medicinal value.

To AMCC: This research will help enhance AMCC's profile in scientific study, showing its support for using local resources in medicinal plant research. It can guide future student researchers in exploring locally available plants.

To the Pharmacy department: This study will add to the department's growing collection of studies on local medicinal plants. It will help the department utilize local medicinal plants in future studies.

1.7 Scope and Limitations

Scope: The study investigated the anti-inflammatory potential of *S. alba* seed extract using a carrageenan-induced paw edema model in mice. It determined the percentage yield of the ethanolic seed extract, conducted toxicity testing, and confirmed its phytochemical composition. The study evaluated the anti-inflammatory potential of different concentrations of *S. alba* ethanolic seed extract (low, mid, and high dose of the median lethal dose) and compared their efficacy to diclofenac sodium.

Limitation: The study was limited to the ethanolic extract of *S. alba* seed and did not explore other parts of the plant. Maceration was the only extraction process used (72 hours). The animal model was also restricted to white albino mice using a specific inflammatory agent (carrageenan). The study only observed the anti-inflammatory effect via the carrageenan-induced paw edema method at time intervals (0, 1, 2, 3, 4, and 5 hours). A phytochemical screening test was conducted to confirm the phytochemical constituents present in the *S. alba* ethanolic seed extract. Lastly, the study only explored whether the *S. alba* ethanolic seed extract has potential anti-inflammatory activity.

1.8 Definition of terms

Anti-inflammatory Agent - Refers to a substance or drug that reduces or prevents inflammation by inhibiting inflammatory mediators such as prostaglandins. This evaluates the potential role of *S. alba* as an anti-inflammatory agent (Bindu et al. 2020).

Carrageenan-induced paw edema model - Refers to a common experimental method used in pharmacology to test the anti-inflammatory properties of a substance. This was used to assess the effectiveness of *S. alba* seed extract compared to the positive control (Kim et al. 2020).

Diclofenac sodium - Refers to a nonsteroidal anti-inflammatory drug (NSAID) used as a positive control in the study (Galge and Thange 2023).

Ethanol - Refers to the solvent of extraction that was used to obtain the ethanolic seed extract of *S. alba*.

Ethanolic Seed Extract - Refers to a solution obtained by using ethanol to pull out chemical compounds from the seeds of *S. alba*. This was used as the main substance in this study to obtain the bioactive compounds for anti-inflammatory evaluation (Lee et al. 2024).

In vivo - Refers to experiments performed on live test animals in which the study employed white mice to evaluate the biological activity of *S. alba* seed extract (Biology Online 2021).

Median Lethal Dose LD50 - Refers to the dose required to cause death in 50% of the test population. It was used to assess the safety of *S. alba* ethanolic seed extract before conducting pharmacological testing (Synapse 2024).

Nonsteroidal Anti-inflammatory Drugs (NSAIDs) - Refers to a class of drugs (including diclofenac) that inhibits cyclooxygenase, which leads to decreased inflammation. They served as the standard comparison to the *S. alba* ethanolic seed extract's effectiveness (Ghlichloo and Gerriets 2023).

Paw Edema - The swelling of an animal's paw induced by carrageenan in this study served as a measurable indicator of inflammation and a parameter to assess the effect of *S. alba* ethanolic seed extract (Kim et al. 2020).

Parenteral Normal Saline Solution - Refers to a sterile solution of sodium chloride (salt) in water that was used as the negative control of the study (Patel et al. 2025).

Phytochemical Profile - Refers to the collection of biologically active chemical compounds, or phytochemicals, found in the *S. alba* ethanolic seed extract. This provided the chemical basis for the potential anti-inflammatory action (Galge and Thange 2023).

Percentage Yield - A calculation that measured the efficiency of an extraction process. It was the ratio of the amount of the final extract product to the amount of the original starting material, expressed as a percentage (Noureen et al. 2023).

Sonneratia alba - Refers to the type of mangrove species used in the study, where its seeds were investigated for potential anti-inflammatory properties (Kulkarni and Manohar 2022).

Seed - Refers to the reproductive structure of a plant containing an embryo, in which the part of *S. alba* was subjected to ethanolic extraction in this study (Biology Online, 2024).

CHAPTER 2
REVIEW OF LITERATURE AND RELATED STUDIES

This chapter provides background information on the concepts of previous studies and the works that directed and provided insights into the investigation of the potential anti-inflammatory activity of *S. alba* ethanolic seed extract in carrageenan-induced paw edema in mice.

2.1 *Sonneratia alba*

Sonneratia alba is a true mangrove species. This evergreen tree is a principal mangrove found in the Indo-West Pacific region. Mangroves are distinctive plants that have long been utilized in traditional medicine. The French botanist and adventurer Pierre Sonnerat (1749–1841) is honored with the genus name Sonneratia. 'White' is the Latin root of the species name alba, which refers to the white stamens and petals of the flowers. *S. alba* (Pagatpat) and mangrove apple have the same common name. The Lythraceae family of flowering plants has 32 genera and over 620 species of trees, shrubs, and herbs. Most members in the Lythraceae family are herbs, although they are also occasionally shrubs or trees, which frequently have flaking bark (Stuart 2024). They possess specialized adaptations and produce various unique metabolites that enable them to survive and flourish in saline and challenging environments (Manohar 2021).

Furthermore, despite the harsh conditions, mangroves have managed to inhabit these environments, developing a range of specialized adaptations such as salt tolerance, viviparous reproduction, and aerial root systems (Feng et al. 2020). The mangroves function as a protector on the coast from abrasion or the brunt of the waves of the tsunami and can maintain the continuity of the population of fish,

shellfish, shrimp, and others (Manuhuttu and Saimima, 2021). *S. alba* are believed to produce up to 200,000 types of secondary metabolites, many with bioactive effects. The amount of secondary metabolite production varies and is determined by genetics and environment. The concentration varies according to the type of vegetation, growth phase, and environmental conditions (Sadeer et al. 2023). For example, mangrove has been considered a new source of drugs, enzymes, nanoparticles, and bioactive compounds which can be applied to food enhancements, nutraceuticals, agriculture, beauty products, and complementary and supplementary medicines (Kumar and Pola 2023).

S. alba typically attains a height of 10–15 m, though shrub-like forms reaching 3 m and large trees growing up to 30 m are occasionally observed. The tree is branched, with cream to brown bark marked by longitudinal fissures. It is surrounded by thick, short, blunt pneumatophores averaging 25–30 cm in length.

The leaves are glabrous, leathery, stalked, simple, and opposite, with an elliptic to obovate shape, generally measuring 5–12 cm in length and 4–6 cm in width. Toward the apex, they become sub-orbicular and obtuse, while the base tapers into a short petiole of 3–5 mm. The species bears bisexual, white flowers (3–6 cm), usually solitary or occasionally in clusters of three. The petals are small and white, with six to eight lobes present within the calyx tube, which is cup-shaped. The sepals are elongated, green externally, and red internally. The stigma is capitate, the style measures about 4 cm, and the ovary is globose. Each flower contains numerous long, showy stamens, 5–8 cm in length, that are white in color. The flowers are nocturnal, blooming in the late evening and lasting for a single night. The fruits are hard, smooth, green, approximately 4 cm in diameter, and contain about 150 small, curved seeds. Fruiting corresponds with the region-specific flowering period, generally occurring between August and February, and the seeds are falcate, dispersed by water (Kulkarnii and Manohar 2024) as illustrated in Figure 2.

2.1.1 Morphology

Figure 2. Photos of S. alba tree (a) Flower (b) leaves (c) and Fruit (d)
Retrieved from Philippine Medicinal Plant (2004), J. B. Friday (2014), Ben Caledonia (2009), and Mangrove_web (2025)

2.1.2 Taxonomy

The taxonomy of *Sonneratia alba (L.)* has been retrieved from the Integrated Taxonomic Information System (ITIS) database (2025).

Kingdom: Plantae
Sub-kingdom: Viridiplantae
Infra-kingdom: Streptophyta
Phylum: Tracheophyta
Sub-phylum: Spermatophytina
Class: Magnoliopsida
Super-order: Rosanae

Order: Myrtales
Family: Lythraceae
Genus : Sonneratia
Species : Sonneratia alba Sm.

2.1.3 Phytochemical content on other parts of the plant

Table 1. Phytochemical content on other parts of the plant

Phytochemical content	Study	Citation
Phytochemical content Alkaloids, Flavonoids, Saponins, Steroids, Tannins and Phenols	According to the phytochemical analysis conducted by Surya et al. (2023), on *S. alba* N-hexane leaf extract showed that phenols and steroids are the confirmed compound present in its leaf. However a study conducted by Sumartini et al. showed that compounds detected on *S. alba* N-hexane leaf extract were alkaloids, flavonoids, saponins, and tannins.	Surya et al. 2023 Sumartini et al. 2022
Flavonoids , Triterpenoids, Steroids,Tannins , and Phenols	Root extracts of *S. alba* were found to contain the following phytochemical compounds : flavonoids, triterpenoids, steroids, tannins, and phenols.	Wijaya et al. 2023
Alkaloids, Triterpenoids, Saponins, Steroids, Tannins and Phenols	The fruit extract contains alkaloids, tannins, saponins, steroids, triterpenoids and phenolics compounds	Dotulong et al. 2024
Flavonoids, Terpenoids, Steroids, Phenols and Tannins	Bark extract of *S. alba* is found to have flavonoids, terpenoids, steroids, phenolics and tannins.	Budia et al. 2019

2.1.4 Ethnobotanical and Pharmacological uses of *S. alba*

The ethnobotanical uses of *S. alba* shows its long-standing role in traditional medicine among coastal communities. Its fruit pulp has been applied to relieve sprains, reduce swelling, and stop bleeding, while preparations from ripe and half-ripe fruits are used to manage intestinal worms, coughs, diarrhea, and other stomach problems. These practices show how the plant has been relied upon to address common ailments using natural remedies. In addition to these traditional applications, modern studies have revealed that *S. alba* contains bioactive compounds responsible for a wide range of pharmacological effects. Research has demonstrated its potential in controlling infections, reducing inflammation, managing blood sugar levels, protecting against oxidative damage, and even showing activity against cancer and malaria.

Table 2. The Summarized Findings of Ethnobotanical uses of *S.alba*

Table 2 shows the ethnobotanical uses of *S. alba* and its role in traditional medicine. The fruit pulp is used as a poultice to reduce swelling and ease sprains, and it can also be applied as a compress to stop bleeding and help wounds heal. Ripe fruits are eaten to remove intestinal worms, while half-ripe fruits are used to relieve coughs. Decoctions and other fruit preparations are taken to treat diarrhea and stomach problems. These practices show that *S. alba* is an important natural resource for managing common health issues in coastal communities.

Ethobotanical Uses	**Details**	**Sources**
Poultice for muscular swellings and sprains	Traditionally, *Sonneratia alba* fruit pulp has been used externally as a poultice to relieve sprains and reduce swelling in the muscles. To reduce pain and inflammation in a number of coastal communities, the softened pulp is mashed and applied directly to the affected areas.	Kulkarnii and Manohar 2024
Compression to stop bleeding (hemorrhage)	Fruit pulp or compress is used topically to check bleeding and as a haemostatic dressing for wounds	Kulkarnii and Manohar 2024
Expulsion of intestinal parasites (antihelmintic)	Traditionally, ripe fruits of *Sonneratia alba* are eaten or prepared into simple remedies to help expel intestinal worms.	Saad et al. 2012
Cough treatment	In traditional ethnomedicinal practice, half-ripe fruits of *Sonneratia alba* are applied to alleviate coughs.	Saad et al. 2012

Treatment for diarrhea	Decoctions or fruit preparations are taken orally to relieve diarrhoea and gastrointestinal upset. This use is reported in local ethnobotanical surveys of mangrove communities.	Kulkarnii and Manohar 2024

This outlined the pharmacological finding of *S. alba*, presenting the plant's Antifungal, Antiplasmodial, Insecticidal, Antioxidant, Antidiabetic, Analgesic, CNS depressant, Tyrosinase Inhibitor, Acetyl cholinesterase inhibitory activity, Cytotoxic, Anti-inflammatory, Anti-atherosclerotic, Anti cancer, Anti bacterial and Anti malarial. These activities contributed to the bioactive compounds present in various parts of the plant, emphasizing for future medicine and treatment use.

Table 3. The Summarized Findings of Pharmacological Use of *S.alba*

Pharmacological Uses	**Title of the study**	**Results/Related Studies**	**Citation**
Antidiabetic	Anti-diabetic polysaccharide from mangrove plant, *Sonneratia alba* Sm. In: Proceedings of the International Conference on Asia Agriculture and Animal	A polysaccharide isolated from the leaf extract of *S. alba* exhibited remarkable blood glucose attenuating activity *In vivo* as it lowered the blood sugar concentration by 19.2% during the first 6 hours and reduced it further to 66.9% post 12 hours of treatment	Morada et al. 2011
Anti-bacterial activity	Antibacterial activity of mangrove Leaf Extract against human pathogens	The anti-bacterial activity of crude methanol extract of bark showed promising activity against eleven tested bacteria. *Staphylococcus aureus, Salmonella typhimurium, Shigella*	Raut and Anthappan 2013

		flexneri, and *Vibrio cholerae* were found to be most susceptible.	
Antioxidant	Antioxidant and antibacterial activities of different extracts and fractions of a mangrove plant *Sonneratia alba*	Ethanol and methanol crude extracts of leaves and bark (water fractions) showed IC50 values 0.019–0.038 mg/ml, close to vitamin C (0.018 mg/ml) in DPPH assay.	Haq et al. 2014
Acetylcholinesterase inhibitory	Anti-cholinergic and anti-microbial properties of *Sonneratia alba* (Perepat)	*In vitro* methanol and dichloromethane leaf and bark extracts showed concentration-dependent inhibition.	Sharudin 2014
Tyrosinase inhibitory	Phenol content, antioxidant and tyrosinase inhibitory activity of mangrove plants in Micronesia	Bark extract had the highest anti-tyrosinase activity (82.4%), followed by leaves (72.5%), roots (40%). Potential for cosmetic applications.	Suh et al. 2014
Analgesic	Analgesic, anti-inflammatory and CNS depressant activities of methanolic extract of *Sonneratia alba* leaves in mice	Methanol leaf fraction (200–400 mg/kg) reduced formalin-induced paw licking in mice.	Asad et al. 2017
Anti-inflammatory	Analgesic, anti-inflammatory and CNS depressant activities of methanolic extract of *Sonneratia alba* leaves in mice.	Methanol leaf fraction (200–400 mg/kg) reduced formalin-induced paw licking in mice.	Asad et al. 2017

CNS depressant	Analgesic, anti-inflammatory and CNS depressant activities of methanolic extract of *Sonneratia alba* leaves in mice	*In vivo* studies showed reduced locomotor activity (sedation).	Siahaya et al. 2018
Anti-atherosclerotic	Triterpenoid compound from methanol extract of mangrove leaves (*Sonneratia alba*) and anticholesterol activity test	Lupeol reduced cholesterol (13.7–77.0%) *In vitro* (5–80 ppm).	Musa et al. 2019
Antimalarial	Antiplasmodial activity of methanolic leaf extract of mangrove plants against *Plasmodium berghei*	Methanol extract (quinone-rich) reduced parasitemia in mice erythrocytes, outperforming pyrimethamine	Muhaimin et al. 2019
Anticancer	The effect of mangrove leaf extract dosage *Sonneratia alba* on HeLa cell viability	Ethyl acetate leaf extract showed moderate cytotoxicity (IC50 = 478.63 µg/ml).	Suryaningrum & Sasmito 2021
Cytotoxic	Inhibitory effect of Malaysian coastal plants on banana, ginger, and sweet potato polyphenol oxidase	Leaf extracts showed toxicity to Artemia salina: LC50 = 3.59 ppm (ethyl acetate), 6.37 ppm (ethanol), 98.37 ppm (n-hexane).	Lim et al. 2021
Antiplasmodial	Antiplasmodial Activity of Ethanol Extract of *Sonneratia alba* Leaves	Ethanol extract suppressed parasitemia dose-dependently: 43.53% (75 mg/kg), 62.35% (150 mg/kg), 77.34% (300 mg/kg).	Muhaimin et al. 2024

2.2 Inflammation

Inflammation is a centuries-old word derived from the Latin inflammare, which means to ignite or burn. An analogy with fire is useful because inflammation's teleological purpose is to 'ignite' in defense against a variety of possible threats until spontaneously extinguishing after threat neutralization (Oronsky et al.,

2022). According to Woodle (2022) Inflammation is the body's reaction to an injury or infection and usually a natural and beneficial part of your body's immune system that aids in the healing process, but it can also contribute to illness formation and progression. Several elements in the modern environment contribute to the onset and persistence of inflammation. These include exposure to environmental contaminants and chemical agents, eating patterns typified by refined carbohydrates, alcohol, and processed foods that disturb the gut microbiota, and unexplained food sensitivities and microbiome dysbiosis. Inadequate sleep, prolonged stress, and a lack of sunlight all contribute to increased inflammatory reactions.

Acute inflammation begins after a specific injury, causing the production of soluble mediators like cytokines, acute phase proteins, and chemokines. These chemicals enhance neutrophil and macrophage migration to the inflammatory site, making them an important component of the innate immune response during acute inflammation (Hannoodee, 2024). Although inflammation is fundamentally a protective mechanism essential for survival particularly in the context of injury and infection and despite evidence that anti-inflammatory treatments can worsen clinical outcomes in sepsis, it should be recognized that inflammation is not a fixed state. Rather, it is a dynamic process that progresses and adapts over time, typically culminating in resolution and the restoration of tissue homeostasis when allowed to proceed naturally (Sundari et al. 2021)

2.3 Treatment for Acute Inflammation

Anti-inflammation treatment also refers to the pharmacological intervention used to reduce excessive immune responses and prevent inflammation induced tissue damage (Chen & Ostermann 2022). Table 4 summarizes the treatments used to manage and relieve symptoms of inflammation by acting as a response modifier to the patient's immune system (Kuchar et. al 2022).

Table 4. Summary of anti-inflammatory treatments

Treatment for anti inflammatory	Description	Citation
Nonsteroidal anti-inflammatory drugs (NSAIDs)	A type of anti-inflammatory drug used to relieve pain, fever and inflammation by inhibiting cyclooxygenase enzymes (COX) that triggers inflammation.	Saad and Mathew 2023
Corticosteroids	An anti-inflammatory drug which acts by inhibiting glucocorticoid receptors that decreases inflammatory activity.	Hodgens and Sharman 2023
Disease-Modifying Antirheumatic Drugs (DMARD)	A class of drug indicated for treatment of several inflammatory Arthritides. Methotrexate and leflunomide fall in this category.	Benjamin et al. 2023

Biologic agents	It is an anti- inflammatory medication that inhibits certain enzymes on the immune system (TNF-α, interleukin-1 and 6) which leads to deactivation of inflammatory pathways mediated by inflammatory factors.	Li et al. 2022
Omega 3 fatty acids	A type of oil found in fish, it can help in treatment for inflammation as it shows an inhibitory activity to pro inflammatory cytokines.	Banaszak et al. 2024

2.4 Acute Oral Toxicity Test

The acute toxic class (ATC) method (TG 423) is an accepted alternative to the LD50 method in 2001. Whilst this test uses fewer animals, death is still the endpoint of assessment. The guideline specifies that three animals (male mice) are tested at fixed dose levels that form the upper limit of the GHS categories (i.e. 5, 50, 300, 2000 mg/kg). The starting dose is either the highest dose, or that which is expected to lead to mortality in some of the exposed animals, based on prior information. At each dose decisions are based on the number of observed deaths from the combined group of animals. Either a classification is made, the test is repeated at the same dose or testing continues at the next higher or lower dose, depending on the starting dose and respective outcomes. (Sewell et al. 2024).

2.5 Carrageenan Induced Paw Edema

The carrageenan-induced paw edema model, introduced by Winter, is one of the most widely used experimental methods for evaluating the anti-inflammatory potential of pharmacological agents (Winter 1962). In this model, acute inflammation is induced by injecting carrageenan into the subplantar region of the rodent paw, producing a biphasic response: an early phase (0–2 h) mediated by histamine, serotonin, and bradykinin, followed by a late phase (3–5 h) characterized by neutrophil infiltration and the release of prostaglandins and cytokines (Di Rosa 1971). Paw swelling is commonly measured using plethysmometry or calipers at different time intervals, and the percentage inhibition of edema is calculated to assess anti-inflammatory activity (Semis et al. 2021).

This model remains a standard due to its simplicity, cost-effectiveness, and reproducibility, making it suitable for screening both synthetic drugs and natural product extracts (Sagar 2020). However, its limitation lies in representing only acute rather than chronic inflammation, and results are often complemented with biochemical or histological analyses to clarify mechanisms (Vinegar 1969). Despite this, the carrageenan induced paw edema assay continues to be a valuable preclinical tool for initial evaluation of anti-inflammatory compounds (Winter 1962)

2.6 Diclofenac Sodium

Diclofenac sodium is an FDA approved drug used in the treatment and management of acute and chronic pain associated with inflammatory conditions, especially those involving the musculoskeletal system. Diclofenac works by blocking the activity of cyclooxygenase enzymes (COX-1 and COX-2). These enzymes are responsible for producing prostanoids such as prostaglandin E2 (PGE2), prostacyclins, and thromboxanes, which play key roles in causing inflammation, pain, and other related responses. These include osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis. Topically, it can treat actinic

keratosis. Diclofenac is also FDA-approved for ophthalmic administration to manage conditions such as cataract extraction, ocular pain, and photophobia. (Alfaro and Davis 2023).

Despite its effects, just like other nonsteroidal anti-inflammatory drugs (NSAIDs), diclofenac sodium can cause side effects such as stomach irritation, peptic ulcers, and decreased kidney function, mostly due to the blocking of prostaglandins. These effects are often unavoidable because they come from how the drug works. However, research shows that diclofenac may cause these problems less often compared to some other NSAIDs. With worldwide use covering more than 7.6 million patient-years, even very rare side effects have been reported. These include blood disorders, liver inflammation (hepatitis), skin reactions like erythema multiforme, as well as rare cases of aseptic meningitis, severe allergic reactions (anaphylaxis), and hives (O'brien WM, 1986)

2.7.Related studies

Plants have been long recognized for their anti-inflammatory properties which are supported by both traditional medicinal use and modern pharmacological studies. Natural medicinal sources are a significant part of research in finding natural and safer alternative synthetic drugs. *S. alba L.* is also known for its anti-inflammatory activity that has been subjected to ethanolic and methanolic extraction.

Table 5. The summarized findings of related study

Table 5 provides a concise summary of published articles, journals, books and other sources studies related to *S. Alba.*

Title of research	Related studies	Citation
Pharmacological Activities of *Sonneratia Alba* Mangrove Plant : A Review.	According to their study the *S. alba* has a potential antimalarial activity and needs further research. It also has antimicrobial activity on several bacteria particularly on *E.coli* which has the highest inhibiting effect. Anticancer and antioxidant activity has also been observed in *S. alba* extract.	Rizki et al. 2023
Antiplasmodial Activity of Ethanol Extract of *Sonneratia alba* Leaves	*S. alba* ethanolic leaf extract showed a significant anti plasmodial activity evident by its ability to decrease the amount of parasitemia levels and inhibit plasmodium growth.	Muhaimin et al. 2024
Antibacterial activity of hexane and methanol fractions of some selected plants against *Klebsiella pneumoniae*	Methanolic leaf extract of *S. alba* had showed a great antimicrobial activity among the other five selected plants against different	Andriani et al. 2023

	pneumonia bacterias	
Phytochemical Screening and Antioxidant Activity Analysis of N-Hexane Extract of *Sonneratia alba* Mangrove Leaves	Based on their study *S. alba* leaf extract showed a strong antioxidant activity with an IC50 value of 64.432 ± 7.675 ppm	Surya et al. 2023
Boiling water extraction of mangrove *Sonneratia alba* fruit as an antioxidant functional food: combined *In vitro* and pharmacoinformatics studies.	*S. alba* sun-dried fruit extract has the best antioxidants activity. It has IC50DPPH = 2.69 ± 0.32 µg/ml compared to vitamin C which has IC50DPPH= 5.04 ± 0.16µg/ml. *S. alba* fruit extract can be a good potential in discovering and developmenting candidates for new antioxidant compounds.	Dotulong et al. 2024
Phytochemicals and Larvicidal Activity of *Sonneratia alba* Root Extracts from Ngurah Rai Mangrove Forest, Denpasar-Bali	*S. alba* extract showed larvicidal activity however it is lower than the positive control that showed a 100%mortality rate at 1ppm compared to *S. alba* extract that showed larvicidal activity at 1265 ppm.	Wijaya et al. 2023

2.8 Theoretical Framework

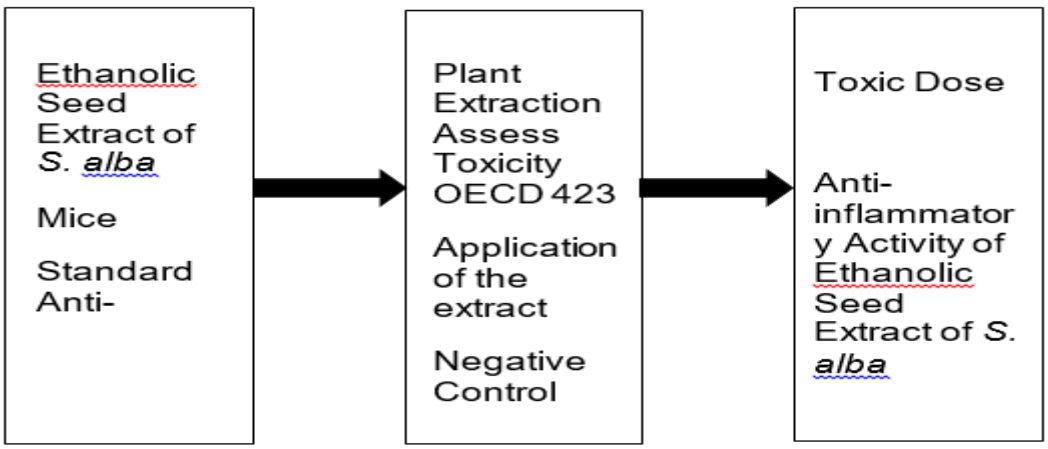


Figure 3. The Theoretical Framework of the Study

The title of our research "The Potential Anti-inflammatory Activity of *Sonneratia alba* Ethanolic Seed Extract in Carrageenan-Induced Paw Edema in Mice" will be following a Theoretical framework. This framework will serve as a guide on how we will administer the ethanolic seed extract of *S. alba* to mice (male) so that we can observe and measure the inflammation. We used this method to find out how the extract works and to measure and collect data about its ability to reduce the inflammation. By controlling the environmental condition and using the type of inflammatory agent, we aim to identify the effect of *S. alba* seed extract on inflammation.

CHAPTER 3
METHODOLOGY

This chapter discusses the methods and materials used for extracting the ethanolic seed extract of *Sonneratia alba*, determining its phytochemical profile, and evaluating the potential anti-inflammatory activity of the ethanolic seed extract of the said plant in carrageenan-induced paw edema in mice.

3.1 Apparatus, Standards, Reagents, Chemicals, and Equipments

The equipment, standards, reagents, and chemicals utilized for the evaluation of the ethanolic seed extract of *S. alba* were purchased from Christian Health Marketing and the AMCC Department of Pharmacy Laboratory. Selected equipment and apparatus that were utilized in the study were from the AMCC Department of Pharmacy and MSU-IIT.

3.2. Authentication, Collection, and Preparation of the Plant Material

A sample of the plant material underwent plant authentication to ensure correct taxonomic identification. A sample of the plant from Cagwait, Surigao del Sur, Philippines were collected for identification. Authentication was requested from the Department of Plant Biology, Mindanao State University–Iligan Institute of Technology (MSU-IIT). A qualified plant botanist examined the morphological characteristics of the specimen, compared them with standard references, and confirmed its scientific identity.

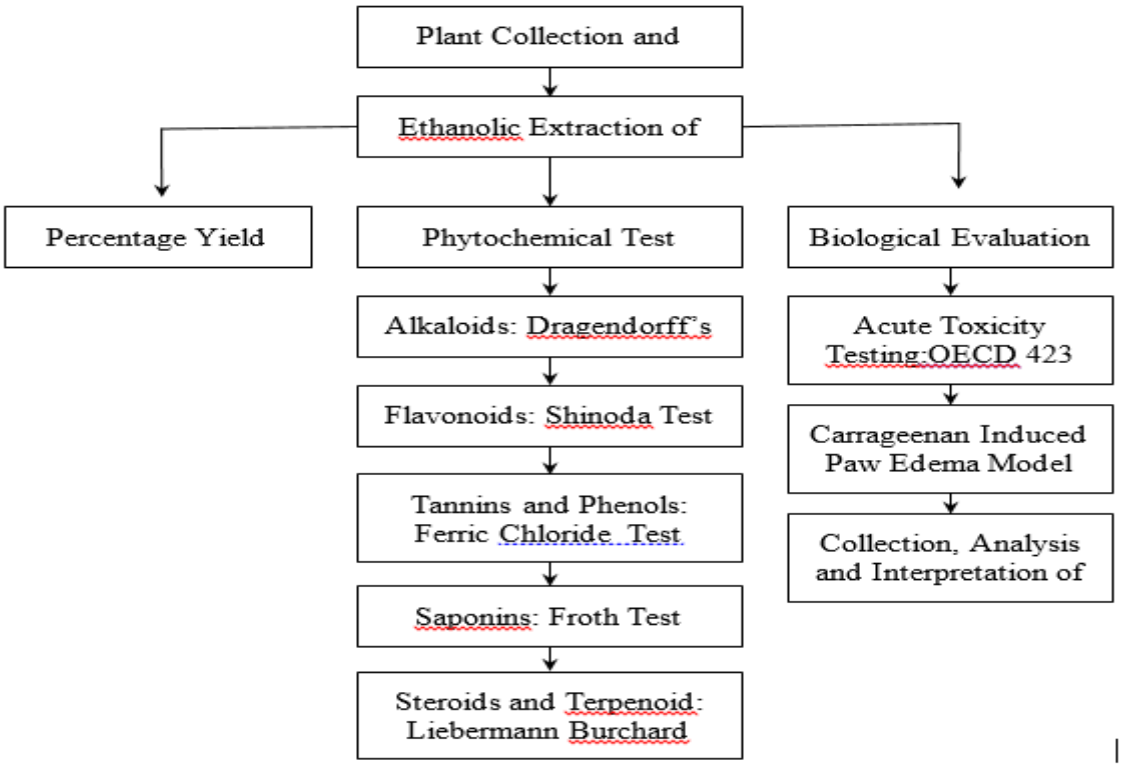


Figure 4. Research Flow of the Study

Figure 4 above shows the general procedures and tests that were conducted in the study.

After authentication, the fruits containing the seeds of *S. alba* were carefully collected from Cagwait, Surigao del Sur, Philippines. Only mature and undamaged fruits were selected to ensure the quality of the seeds as raw material. The seeds of *S. alba* were collected from the fruits of the plant, and only seeds that were free from bruises and signs of plant disease were carefully selected and hand-picked. The seeds were then thoroughly washed with distilled water to remove any dirt and impurities. They were air-dried at room temperature (20–25 °C) for two to three weeks until fully dried. The dried seeds were ground into a fine powder using a blender (Ninja Foodi SS201 Power Blender & Processor). The pulverized seeds were then passed through a 120-mesh sieve with a particle size of 125 µm.

The pulverized seeds were kept in an airtight plastic ziplock bag, stored in a cool, dry, and shaded area away from sunlight at 4°–25°C to prevent degradation of phytochemical content. The pulverized seeds were stored until use for percentage yield analysis and biological test evaluation.

One part of the pulverized plant material was macerated with five parts of 95% ethanol in an Erlenmeyer flask. The mixture was continuously stirred using an orbital shaker at a speed of 100 rpm for a duration of 72 hours for the extraction of bioactive compounds into the solvent. After maceration, the mixture was filtered using ordinary filter paper and a glass funnel to separate the liquid extract from the plant residue. The liquid extract was concentrated using a water bath at a constant temperature of 40°C to remove the ethanol content and obtain the crude extract. Afterwards, the semi-solid crude extract was transferred into a clean amber-colored container and stored in a refrigerator at 2–4°C until further use (Casiple et al., 2025).

3.3 Percentage Yield Analysis

Extraction yield was determined via triplicate analysis of a 10-gram sample of *S. alba* powdered seeds subjected to ethanolic extraction using 95% ethanol following a 1:5 ratio of extraction (1 gram of the pulverized seed soaked in 5 mL of 95% ethanol). The percentage yield was calculated as the weight of the obtained crude extract divided by the weight of the powdered sample, multiplied by 100.

Calculation of yield results was done using the formula below (Suryaningrum 2021):

$$\text{Yield of Extract \%} = \left(\frac{Weight\ of\ crude\ extract\ (g)}{Weight\ of\ powdered\ sample(g)}\right) \times\ 100$$

3.4 Phytochemical Screening Test

Phytochemical screening were conducted to determine the phytochemical profile of the ethanolic seed extract of *S. alba* using standard tests and reagents to confirm the presence of secondary metabolites, specifically flavonoids, alkaloids, tannins, saponins, phenolics, steroids, and terpenoids. The presence of these selected phytochemical compounds in a plant suggested potential anti-inflammatory activity. For each phytochemical test, 5 mg of the ethanolic seed extract were utilized. Table 6, presented the phytochemical screening test procedures used to detect major phytochemicals in the 95% ethanol extract of *S. alba*. Phytochemical screening was performed on the ethanol extract of *S. alba* using different chemical reagents.

Table 6. Phytochemical Screening Test Procedure for 95% of Ethanol Extract from *S. alba*

Phytochemical/s	**Test**	**Expected Positive Result**	**Procedure**
Alkaloids	Dragendroff's	Orange to red brown	One ml of Dragendorff's reagent was

	test	color	added to the sample solution. If an orange to red brown color was observed, it indicated the presence of alkaloids compounds (Sumartini et al. 2022).
Flavonoids	Shinoda test	Pink or yellow color	0.05 mg of Shinoda's reagent and 1 ml of HCl were added to the 5 ml plant sample, and it was shaken vigorously until it was homogenized. Color change was observed (Sumartini et al. 2022).
Test for Tannins and Phenols	Ferric chloride test	Brown precipitation	5% Ferric chloride (FeCl3) reagent was added to the 1ml plant sample (Sumartini et al. 2022).
Saponins	Froth Test	Formation of stable foam above the solution.	Two mL of distilled water was added to the plant sample and was shaken for a few minutes (Sumartini et al. 2022).
Steroids and Trapenoid	Liebermann Burchard	Blue to green for steroids and brown to reddish brown for trapenoids.	3.5 drops of chloroform were added to 1 ml of the plant extract. Then, 3 to 5 drops of acetic anhydride and 10 drops of concentrated sulfuric acid were added subsequently (Sumartini et al. 2022).

3.5 Acute Oral Toxicity Testing: Acute Toxic Class Method (OECD 423)

3.5.1 Selection Animals

Healthy male mice were utilized since they have more stable hormone levels than females, allowing for reliable results. Mice that would be used will be six to eight weeks old weighing between 20 to 35 grams mice were utilized. To maintain uniformity, each mouse selected 20±% of the average group. (Casiple et al 2025)

3.5.2 Housing and Feeding Condition

The temperature in the experimental animal room was 22ºC (± 3ºC). The relative humidity was at least 30% and preferably did not exceed 70%, except during room cleaning; the aim was 50–60%. Lighting was artificial, with a sequence of 12 hours light and 12 hours dark. For feeding, conventional laboratory diets (store-bought pellets) were used with an unlimited supply of drinking water (OECD 2001). Test mice were selected at random and labeled to enable individual identification in a randomized controlled trial that followed the study (Casiple et al. 2025).

3.5.3 Acute Oral Toxicity Testing: Acute Toxic Class Method (OECD 423)

The acute toxic class (ATC) method (TG 423) was utilized since it is an alternative to the LD50 method. Although this test utilizes fewer animals, death still served as the endpoint of assessment (Sewell et al. 2024). The test animals were required to fast for three to four hours, but they had free access to water. After fasting, the mice were weighed, and a single dose of the ethanolic seed extract of *S. alba* was given

by oral gavage to randomly selected male albino mice; three animals were used for each step (Casiple et al. 2025).

The starting dose level was chosen from one of four fixed levels: 5, 50, 300, or 2000 mg/kg body weight. The first dose was the one most likely to produce mortality in some of the animals, which was 2000 mg/kg. The time gap between treatment groups depended on the onset, duration, and severity of toxic signs. Treatment at the next dose level was delayed until survival of the previously dosed animals was confirmed (OECD 2001). If the ethanolic seed extract of *S. alba* showed toxic effects, the doses were lowered in the next test to ensure safety and to establish a more accurate toxicity threshold.

After administering the ethanolic seed extract of *S. alba*, the animals were observed individually after dosing, at least once during the first 30 minutes, then periodically during the first 24 hours, with special attention during the first 4 hours, and daily thereafter for a total of 14 days. However, the observation period was not fixed strictly. It was based on the toxic reactions, time of onset, and length of recovery, and could be extended if necessary. The times when toxic signs appeared and disappeared were noted, especially if signs were delayed. All observations were recorded systematically, with individual records kept for each animal (OECD 2001). Lastly, gross necropsy was performed on the diseased mice to evaluate internal and external conditions and abnormalities.

3.5.4 Administration of Test Substance

A dose of 2000 mg/kg body weight of the ethanolic extract of *S. alba* were given to the test animals. They will undergo a four-hour fasting period before the experiment begins. Three animals (n=3) were included in the experimental group, each receiving the test substance orally through gavage as a single dose. Following administration, the animals will remain without food for another two hours. The entire procedure was carried out in accordance with the OECD 423 (2023) guidelines.

3.6 Evaluation for Anti-inflammatory Activity: Carrageenan-Induced Paw Edema Method

The anti-inflammatory activity was evaluated by using the carrageenan-induced paw edema model in mice. This model is commonly used to assess inflammation in in vivo studies. Paw edema was induced by subplantar injection in the hind paw of the mouse. The progression of inflammation was monitored over a specified period of time (0, 1, 2, 3, 4, 5 hours). The degree of decreased inflammation, which was indicated by a decrease in paw swelling, served as the primary indicator for evaluating the anti-inflammatory effect of the test compound.

3.6.1 Ingredients and Procedure of Carrageenan

Carrageenan was commonly used to induce paw edema in experimental mice models, where a 1% suspension was prepared from carrageenan powder and 0.9% sterile saline solution, which served as the substance that induced inflammation (Cabrera et al. 2021). Table 7 showed the ingredients used to make the carrageenan suspension, which consisted of 0.9% saline solution and carrageenan powder. The 1% carrageenan was induced in paw edema of Swiss mice. To make the 1% carrageenan sterile solution, 1 g of carrageenan powder was dissolved into 100 mL of 0.9% sodium chloride sterile solution inside a 250 mL beaker. Then the mixture was heated to 40–50ºC and constantly stirred for 15–30 minutes until the carrageenan powder dissolved completely, resulting in a clear and slightly viscous liquid. It was then cooled at room temperature (15–30ºC). The solution was then injected into the hind paw of mice subcutaneously using a sterile needle. The control paw of the mice was measured at time intervals, every hour for 5 hours, starting at 0 hour as the baseline (Casiple et al. 2025).

Table 7. Ingredients of Carrageenan Solution for Paw-Edema Induction

Ingredients	Measurement
0.9% Sterile Solution	100 mL
Carrageenan powder	1g

3.6.2 Selection of Animals

In this study, male mice that were six to eight weeks old were utilized. For these mice to be healthy and receptive to therapies, their ideal weight was between 20 and 35 grams. This combination decreased result variability and allowed for an efficient evaluation of the extract's therapeutic potential (Casiple et al. 2025).

3.6.3 Housing and Feeding Condition

The temperature in the experimental animal room was maintained at a constant 22ºC (± 3ºC). The relative humidity was at least 30% and preferably did not exceed 70%, except during room cleaning, with the aim of around 50–60%. Lighting was artificial, with a sequence of 12 hours light and 12 hours dark. For feeding, conventional laboratory diets (pellets) were used with an unlimited supply of distilled drinking water (OECD 2001). Test mice were selected at random and labeled to enable individual identification in a randomized controlled trial that followed the study (Casiple et al. 2025).

3.6.4 Selection of Doses

The anti-inflammatory assay was administered at low, medium, and high doses based on the acute oral toxicity (OECD 423). The test animals were fasted for four hours before the experimentation with free access to water. This ensured that recent food intake would not interfere with drug absorption, distribution, and anti-inflammatory response (Asih et al. 2025). Three treatment levels corresponding to low, mid, and high doses of the LD_{50} were used for the anti-inflammatory experiment. The experimental groups included a negative control treated with normal saline solution (NSS 10 mL/kg) and a positive control receiving diclofenac sodium (10 mg/kg).

Table 8. Doses to be Administered to Evaluate the Anti-inflammatory Activity of *S. alba*

Group	Treatment	Dose	Route of administration	No. of Animals
Group I	*Sonneratia alba* Ethanolic Seed Extract	Low dose of acute toxic dose	Oral (p.o)	3
Group II	*Sonneratia alba* Ethanolic Seed Extract	Middle dose of acute toxic dose	Oral (p.o)	3

Group III	*Sonneratia alba* Ethanolic Seed Extract	High dose of acute toxic dose	Oral (p.o)	3
Group IV	Control (NSS)	10ml/kg	Oral (p.o)	3
Group V	Diclofenac Sodium	10ml/kg	Oral (p.o)	3

The carrageenan-induced hind paw edema model was utilized to evaluate the anti-inflammatory activity of *S. alba* ethanolic seed extract, following the method described by OECD (2001). The mice were randomly divided into five groups, consisting of three mice per group. Using a digital vernier caliper, the progression of paw edema was assessed at 0, 1, 2, 3, 4, and 5-hour intervals. The animals were pre-treated with *S. alba* for one hour before receiving the carrageenan injection at doses determined through acute oral toxicity testing for the first three groups. Diclofenac sodium was administered as the reference medication, and 0.9% sodium chloride as a control, to the other two groups (Casiple et al. 2025).

3.6.5 Induction of Inflammation through Prepared Carrageenan Solution

To cause paw inflammation and edema, mice in each group received a 0.1 ml injection of a freshly prepared 0.1% carrageenan suspension into their right paw. The left hind paw was used as a control to determine the change in paw thickness and was not treated. It was anticipated that carrageenan would result in noticeable redness and significant swelling within three hours, which would last until the end of the experiment (Casiple et al. 2025).

3.6.6 Time Intervals for Measurement

The paw thickness was measured every hour for a period of five hours, starting at 0 hour, which was measured immediately after induction of carrageenan in the paw of the mice. The paw thickness was measured using a digital vernier caliper at 0, 1, 2, 3, 4, and 5 hours after carrageenan injection. Changes in paw thickness relative to the baseline (0 hour) were recorded to evaluate the anti-inflammatory effects of the treatments. The results of paw edema were expressed as the change in paw thickness at each time point relative to the initial measurement at time zero (Narimani Zamanabadi et al. 2025).

3.6.7 Measurement of Paw Thickness

The paw thickness was measured using a digital vernier caliper prior to injection of carrageenan, which was used as the baseline measurement. The paw was measured again at 1, 2, 3, 4, and 5 hours post-injection. The edema was measured as the increase in paw thickness relative to the baseline (Narimani Zamanabadi et al. 2025).

3.6.8 Calculation of Percentage Edema

The percentage edema was calculated using the formula:

$$\%\ of\ inhibition\ = \frac{(Vcontrol\ -\ V\ treated)}{Vcontrol} x\ 100\%$$

Where:

Vt = paw volume at a given timepoint

Vc = initial volume before carrageenan injection .

This formula compared the volume of edema between the control group and treated group over time. It directly reflected the progression of inflammation in each animal over time (Kuang et al. 2021).

3.7 Data analysis

All data were expressed as mean + (SEM) standard error of the mean. Statistical differences among groups were analyzed using one-way ANOVA, Tukey's post hoc test, and linear regression. Repeated measurements were analyzed using one-way repeated ANOVA (Ramili et al. 2025). Statistical significance was considered at $p < 0.05$, and analysis was performed using GraphPad Prism or similar statistical software.

CHAPTER 4
RESULT AND DISCUSSION

This chapter provided the results and discussions from the methodology provided in the previous chapter. This includes the descriptions, percentage yield, phytochemical screening findings, acute toxicity test, and descriptive results of different concentrations of *S. alba* 95% ethanolic seed extract concentrations (low, mid, and high), LD_{50} dosage, positive control (Diclofenac sodium), negative control (NSS), effective dose, and data analysis. Furthermore, relevant interpretations and discussions are presented to provide a thorough understanding of the observed results and their implications in connection to the study objective.

4.1 Extraction of the Plant Extract

The crude ethanolic seed extract of *S. alba* appears as a thick, dark brown and it has dense, viscous, paste-like consistency. The extract was obtained through a solvent based extraction procedures, where a solvent was used to dissolve the powdered seed of *S.alba* using ethanol, it yields a concentrated solution that is rich in bioactive compounds such as alkaloids, total phenolic, tannins, saponins, flavonoids, terpenoids and steroids.

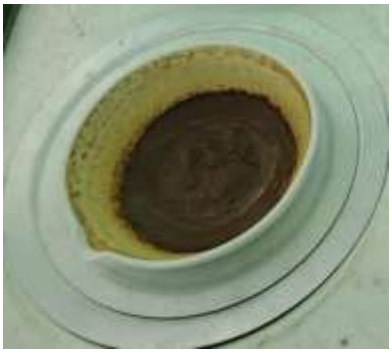


Figure 5. *S. alba* crude extract

4.2. Percentage yield analysis

The average percentage yield was determined from three different trials of gram samples, which had a computed mean of 64.507%. The table 9 shows the individual yield obtained from each trial and the average percentage yields of the crude ethanolic seed extract of *S. alba* is determined by using the formula below:

$$\text{Yield of extract \%} = \left(\frac{Weight\ of\ crude\ extract\ (g)}{Weight\ of\ powdered\ sample(g)}\right) \times 100$$

Table 9. Percentage Yield of the crude ethanolic seed extract of *S. alba*

Trial	Sample	Crude ethanolic extract *S. alba*	Percentage Yield
1	10g	5.385 g	53.85 %
2	10g	7.569 g	75.69%
3	10g	6.398 g	63.98%
Mean value			64.507% ± 10.93

Table 9 presented the ethanolic seed extract of *Sonneratia alba* obtained from three different samples. The weight of powdered dried seed used for each sample was 10 g and the corresponding weight of the crude extract obtained with the calculated percentage yield based on the weight of the dried seeds presented. The percentage yield ranged from 53.85% to 75.69%, while the crude extract weights ranged from 5.385 g to 7.569 g having the mean value of 64.507%. The extraction yield was determined through triplicate analysis using 95% ethanol as the extraction solvent following a 1:5 extraction ratio. After maceration and filtration, the solvent was evaporated using a water bath at 40°C to obtain the crude extract. The extract obtained showed a thick, dark brown, dense, viscous, and paste-like consistency, indicating that the ethanol solvent had been successfully concentrated, leaving behind bioactive compounds present in the seed extract. The variation in percentage yield among the three trials was attributed to differences in extraction efficiency, solvent penetration, and the amount of phytochemical constituents present in each sample.

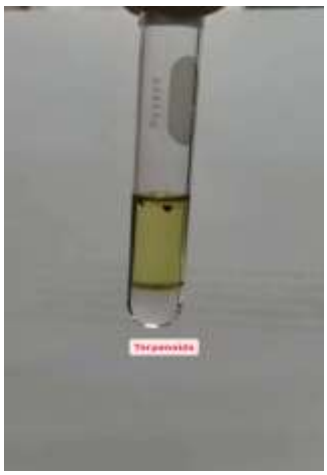
4.3. Phytochemical Screening Result of *S.alba*

A phytochemical screening test was conducted at MSU-Iligan Institute of Technology (MSU-IIT) by Miss Enjelyn Calleno Gomez, a licensed chemist of the institution, to determine the presence of selected phytochemical constituents in the sample. The analysis focused on the detection of alkaloids, flavonoids, phenols, saponins, steroids, tannins, and terpenoids.

Table 10. Phytochemical constituents present in *S.alba* ethanolic seed extract.
The results of the phytochemical screening are presented below, including the specific tests performed, corresponding visual observations, and their respective interpretations.

Phytochemical Test	Visual Result	Interpretation
Test for alkaloids (Wagner's Test)		The formation of a reddish-brown coloration in Wagner's Test for Alkaloids indicates a positive result, confirming the presence of alkaloids in the sample.

Test for Flavonoids (Shinoda Test)		The development of a dark reddish coloration in the left test tube during the Flavonoids test indicates a positive result, confirming the presence of flavonoids in the sample.
Test for Phenolics (Ferric Chloride Test)		The formation of a dark blue to black coloration in the left test tube during the Phenolic compounds test indicates a positive result, confirming the presence of total phenolics in the sample.
Test for Saponins (Froth Test)		The appearance of a stable frothy layer measuring at least 1 cm in the left test tube during the Saponins test indicates a positive result, confirming the presence of saponins in the sample.
Test for Steroids (Liebermann Burchard Test)		The sample shows an orange-yellow liquid with a dark reddish-brown layer concentrated at the

		bottom. This color pattern indicates a positive reaction and confirms the presence of steroid compounds in the sample.
Test for Tannins (Ferric Chloride Test)		The sample shows a dark blue-black solution with a thin light layer at the top. This color change indicates a positive reaction, confirming the presence of tannins in the sample.
Test for Terpenoids (Liebermann Burchard Test)		The formation of a yellowish solution with a distinct layer separation in the Terpenoid Test indicates a positive result, confirming the presence of terpenoids in the sample

The presence of these phytochemical constituents is consistent with findings commonly reported in medicinal plants. Compounds such as flavonoids, tannins, saponins, and steroids are known for their diverse biological activities, including antioxidant, antimicrobial, and anti-inflammatory properties. The

detection of these constituents in *S. alba* suggests that the plant has significant therapeutic potential and could serve as a valuable source of bioactive compounds.

Table 11. Phytochemical Constituent Detected in *S. alba* Seed Extract

Phytochemical Constituent	**Result**
Alkaloids	Positive
Flavonoids	Positive
Phenols	Positive
Saponins	Positive
Steroids	Positive
Tannins	Positive
Terpenoids	Positive

The table above presents the results of the phytochemical screening of the *S. alba* crude extract. The findings showed positive results for the presence of alkaloids, flavonoids, phenols, saponins, steroids, tannins, and terpenoids in the extract. A positive result indicates that the phytochemical constituent was detected during the qualitative screening test..

4.4. Isolation of Mice

The experimental mice used in this study were properly isolated and housed under controlled laboratory conditions prior to and during the conduct of the experiment. Healthy male Swiss albino mice, aged 6–8 weeks and weighing approximately 20–35 grams, were selected to ensure uniformity and reliability of results (Casiple et al., 2025). Each mouse was housed in clean, well-ventilated cages, with bedding materials replaced every other day and cages cleaned regularly. The animals were bathed once a week to maintain proper hygiene. The animals were treated according to their respective experimental treatments. Furthermore, all cages were properly labeled with the corresponding weight and assigned dose of each animal for accurate identification and monitoring throughout the study. Environmental conditions in the departmental animal house were maintained under standard laboratory settings. The temperature was strictly controlled at 22–25°C, and a 12-hour light–dark cycle was observed. The mice were provided with a standard laboratory diet (pellets) and an unlimited supply of drinking water *ad libitum* (Sun et al., 2021). Isolation also involved minimizing external disturbances such as noise, excessive handling, and exposure to harmful substances that could affect the physiological response of the animals. During the experimental phase, each group (negative control, positive control, and treatment groups with varying concentrations of *Sonneratia alba* ethanolic seed extract) was handled separately to prevent bias and ensure the integrity of the results. Proper animal care and handling procedures were strictly followed in accordance with ethical guidelines for laboratory animal use (OECD, 2001). This ensured that the isolation conditions supported both the welfare of the animals and the reliability of the experimental outcomes.

4.5 Acute Oral Toxicity Test

The acute toxicity test began with four groups of mice, with each group consisting of three mice. The first group was subjected to a dose of 5 mg/kg, the second group was administered a dose of 50 mg/kg, the third group received a dose of 300 mg/kg, and the fourth group received a dose of 2000 mg/kg. No abnormal behavior nor mortality was observed during the first 24 hours after administration. Following the OECD guidelines, 5000mg/kg was administered to a fifth group of three mice. No behavioral changes and death were observed after 24 hours. However, during the seventh day one mouse died under the 5000mg/kg dose (refer to appendix L and O). A gross necropsy was conducted to the dead mouse, we observed that its intestines were swollen or appeared bloated. No other organs showed any visible signs of damage or toxicity, indicating that the issue seemed to be limited to the intestines only. The rest of the mice were continuedly observed until the fourteenth day, during which no visible signs of toxicity such as tremors or physical and behavioral changes were observed. Based on these findings the median lethal dose of *S. alba* extract was estimated to be greater than 5000mg/kg.

Table 12. Graded Dose levels of *S. alba* ethanolic seed extract following OECD 423

Dose level	**Actual dose (mg/kg)**
Low Dose	50mg
Middle Dose	300mg
High Dose	2000mg

The table presents the graded dose levels of *S. alba* ethanolic seed extract administered to the experimental groups during the acute toxicity study. A low, mid, and high dose scheme was used, following the dosing guidelines for mice based on OECD guidelines. Specifically, the low dose was 50 mg/kg, the mid dose was 300 mg/kg, and the high dose was 2000 mg/kg.

4.6 Evaluation of Test Result

The evaluation of the test result was conducted to determine the anti-inflammatory efficacy of the 95% ethanolic seed extract of *S.alba* using acute edema model. The findings were analyzed based on the degree of reduction of paw edema across the different treatment groups and time intervals, in comparison to the negative and positive control groups.

4.6.1 Anti-inflammatory Activity: Carrageenan-Induced Paw Edema

The anti-inflammatory potential of *S. alba* ethanolic seed extract was evaluated using the carrageenan-induced hind paw edema model in male Swiss albino mice. The animals were grouped into negative control (PNSS), positive control (Diclofenac sodium) 10 mg/kg, and treatment groups receiving low, mid, and high doses of the extract based on the LD_{50}. Paw edema was measured from the initial reading up to the 5th hour to monitor the progression of inflammation. (Graph figure 6 and 7) illustrates trends in inflammation.

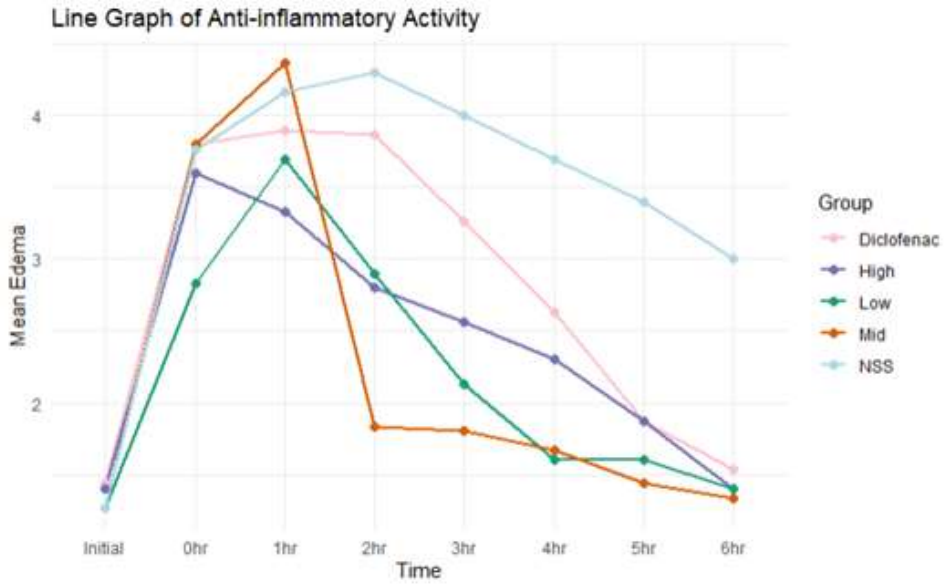


Figure 6 Line graph result of *S. alba* Ethanolic Seed Extract (low, mid, high dose) and the control groups (positive and negative)

Figure 6 (line graph) presents the mean edema values for each dose level across all time points and illustrates the progression of the carrageenan-induced inflammatory response. As observed in the illustration above, edema increased from Initial to the 1st hour, indicating onset and peak of inflammation then followed by a gradual decline from the 2nd hour to the 5th hour reflecting the resolution phase of inflammation. The mid dose (represented by red line) shows the highest peak edema at the 1st hour, while the high dose (represented by purple line) did not consistently produce the greatest reduction in edema. The low dose (represented by green line) exhibits comparable reduction than higher doses. Furthermore, the anti-inflammatory response was measured at regular intervals from the initial measurement to the 5th hour. The x-axis in the graph indicates the following time points: initial (before carrageenan injection), 0 hour, 1st hour, 2nd hour, 3rd hour, 4th hour, and 5th hour, which indicate the growth of inflammation and therapeutic effects over time. At the start, all groups had equal baseline paw volumes, indicating consistent pre-treatment conditions. Following carrageenan injection (0 hour), a marked increase in paw edema was observed across all groups, confirming successful induction of inflammation. The negative control group (represented by blue line) consistently showed the highest edema values throughout the observation period, particularly peaking between the 1st and 2nd hour, which indicates the absence of anti-inflammatory activity. In contrast, the positive control (represented by the pink line) showed a progressive decline in paw edema following the peak inflammation, in particular between the 3rd and 5th hours, confirming its predicted anti-inflammatory effect. Additionally, the groups treated with *S. alba* extract reduced edema beginning with the 2nd hour, indicating the presence of anti-inflammatory action. Among the treatment groups, the mid dosage had the most edema at the 1st hour, suggesting a higher initial inflammatory response, but the low and high doses showed more consistent decreases at subsequent time points. By the fifth hour, all treated groups showed decreased paw volumes than the negative control. However, the reduction in edema was not absolutely dose-dependent, since the high dosage did not have the greatest reduction of edema compared to the low dose which showed similar effects in the succeeding hours. The differences among extract doses were also not consistent across all time points, and no clear

dose-response relationship was observed. Despite this, the treated groups significantly differ from the negative control at later time intervals, indicating that the extract effectively reduces inflammation over time. Overall, the line graph supports that *S. alba* ethanolic seed extract has time-dependent anti-inflammatory activity, although its effect varies across doses and was less potent compared to the positive control.

4.6.2 Descriptive Result

The carrageenan-induced hind paw edema model was applied to assess the anti-inflammatory activity of 95% ethanolic seed extract of *S. alba* (Lythraceae). Male swiss albino mice were used and were randomly selected and grouped into five, in each group it consisted of three animals.

Table 13. Result of Positive Control (Diclofenac Sodium) with mean and standard deviation.

Positive Control	Mean	Standard Deviation
Initial	1.433333	0.3214550
0 hr	3.800000	0.4582576
1st hr	3.900000	0.3605551
2nd hr	3.866667	0.3511885
3rd hr	3.266667	0.3214550
4th hr	2.633333	0.5507571
5th hr	1.866667	0.1527525
6th hr	1.533333	0.2516611

Table 13 served as a positive control and was treated with diclofenac sodium, a standard anti-inflammatory drug. It shows in the table above the mean paw volume of this group from initial paw measurement 1.433 mm (±0.321) and it increased in the 0 hr 3.800 mm (±0.458). At the 1st hour, edema value further increased 3.900 mm (±0.360), but in the 2nd hour there is a decrease in edema 3.866 (±0.311). In the 3rd hour, it continued to decrease 3.266 mm (±0.321), and a more observable decrease at the 4th hour 2.633 mm (±0.550). By the 5th hour, the edema was still decreasing 1.866 mm (±0.152), and lastly in the 6th hr, the edema was near baseline level 1.533 mm (±0.251). These result shows that the diclofenac sodium reduce the carrageenan-induced inflammation over time, as it shows in the initial to 1st hour, there is no significant differences, but in the 2nd hour until 6th hour there is a significant difference, showing the onset of anti-inflammatory activity of *S. alba.*

The anti-inflammatory activity of the 95% ethanolic leaf extract of *S. alba* (Lythraceae) was evaluated using the carrageenan-induced hind paw edema model. Male Swiss albino mice were randomly assigned into five groups, with three animals in each group. One group was designated as the negative control and received Normal Saline Solution (NSS), which has no anti-inflammatory effect.

Table 14. Result of Negative Control (PNSS) with mean and standard deviation.

Negative control	Mean	Standard Deviation
Initial	1.266667	0.1527525
0 hr	3.766667	0.1527525
1st hr	4.166667	0.1527525
2nd hr	4.300000	0.1000000
3rd hr	4.000000	0.1000000
4th hr	3.700000	0.1000000
5th hr	3.400000	0.1000000
6th hr	3.000000	0.1000000

Table 14 served as a negative control Normal Saline Solution (NSS) was used as a treatment. The table above shows the mean value of paw in this group, from initial paw measurement 1.266 mm (± 0.152), at 0 hour it increased to 3.766 mm (±0.152). During the 1st hour it rose to 4.166 mm (±0.152), then at the 2nd hour it peaked at 4.3 mm (±0.100), upon the 3rd hour it decreased at 4.00 mm (±0.100) and further declined in 4th hour by 3.7mm (±0.100), followed by 3.40mm (±0.100) in 5th hour, by the 6th hour the paw measures 3.00mm (±0.100). These results indicate a slight but steady decline of inflammation over time in the negative control group.

The anti-inflammatory activity of the 95% ethanolic leaf extract of *S. alba* (Lythraceae) was assessed using the carrageenan-induced hind paw edema model. Male Swiss albino mice were randomly selected and assigned into five groups, with three animals in each group. One of the treatment groups was given a high dose of the *S. alba* extract, corresponding to 2000 mg/kg based on its acute toxicity level.

Table 15. Result of *S. alba* Ethanolic Seed Extract (high dose of 2000 mg/kg of its acute toxic dose) with mean and standard deviation.

High Dose (*S. alba* 2000 mg/kg)	**Mean**	**Standard Deviation**
Initial	1.400000	0.1000000
0 hr	3.600000	0.4358899
1st hr	3.333333	0.1154701
2nd hr	2.800000	0.6082763
3rd hr	2.566667	0.5859465

4th hr	2.300000	0.6082763
5th hr	1.866667	0.1527525
6th hr	1.400000	0.1000000

As presented in Table 15, At baseline, the high dose group showed a mean paw edema of 1.400 (± 0.100), in the 0 hour, the mean edema increased to 3.600 mm (± 0.435), reflecting the successful induction of inflammation. By the 1st hour, the edema slightly decreased to 3.333 mm (±0.115), but remained relatively high, indicating the peak phase of inflammation, At the 2nd hour, the mean edema decreased to 2.800 mm (±0.608), showing a clear reduction compared to earlier time points. In the 3rd hour it further decreased to 2.566 mm (±0.585), while in the 4th hour, the edema still decreased 2.300 mm (±0.608). Observing in the 5th hour, the edema decreased to 1.866 mm (±0.152) as it nears the baseline level, and lastly, the 6th hour, the mean edema returned to 1.400 mm (±0.100), which nearly identical to the initial value. The high dose of *S. alba* ethanolic seed extract exhibited a gradual and sustained reduction in paw edema over time. While no immediate effect was observed during the early phase (0–1 hour), a noticeable anti-inflammatory effect began at the 2nd hour and continued progressively until the 6th hour, where edema returned to near baseline levels. These findings indicate that the high dose extract has significant anti-inflammatory activity, particularly during the later stages of inflammation (4th, 5th and 6th hour).

Table 16. Result of *S. alba* Ethanolic Seed Extract (Mid Dose of 300 mg/kg of its acute toxic dose) with mean and standard deviation.

Mid Dose (*S. alba* 200 mg/kg)	**Mean**	**Standard Deviation**
Initial	1.266667	0.2081666
0 hr	3.800000	0.3464102
1st hr	4.366667	0.3511885
2nd hr	1.833333	0.3785939
3rd hr	1.800000	0.3605551
4th hr	1.666667	0.2516611
5th hr	1.433333	0.2516611
6th hr	1.333333	0.1527525

As indicated in Table 16, At baseline, the mid dose group showed a mean paw edema of 1.266 mm (±0.208), At 0 hour, the mean edema sharply increased to 3.800 mm (± 0.346), indicating the successful induction of inflammation. At the 1st hour, the edema further increased to 4.366 mm (±0.351), in the 2nd hour, the mean edema markedly decreased to 1.833 mm (± 0.378). In the 3rd hour, the edema slightly

decreased further to 1.800 mm (±0.360), 4th hour, the mean edema was 1.666 mm (±0.251), showing continued gradual decline of inflammation. At the 5th hour, the edema decreased further to 1.433 mm (±0.251), approaching baseline levels. In the 6th hour, the mean edema reached 1.333 mm (±0.152), which was very close to the initial value. The mid dose of *S. alba* ethanolic seed extract shows a distinct pattern of anti-inflammatory activity. While it showed the highest peak inflammation at the 1st hour, it also demonstrated the most rapid and pronounced reduction in edema starting at the 2nd hour. The effect was sustained throughout the succeeding time points, leading to near-complete resolution by the 6th hour. This suggests that the mid dose may represent an optimal concentration, providing a strong and consistent anti-inflammatory effect.

Table 17. Result of *S.alba* Ethanolic Seed Extract (Low dose of 50mg/kg of its acute dose) with mean and standard deviation.

Low dose (*S. alba* 50 mg/kg)	**Mean**	**Standard Deviation**
Initial	1.266667	0.2081666
0 hour	2.833333	0.7637626
1st hour	3.700000	1.1532563
2nd hour	2.900000	0.4000000
3rd hour	2.133333	0.6350853
4th hour	1.600000	0.4582576
5th hour	1.600000	0.3464102
6th hour	1.400000	0.1732051

Based on the table 17, the starting paw measurement is 1.266 mm (± 0.208), at 0 hour it increased to 2.83 mm (± 0.766), with in its 1st hour it hit its peak with a measurement of 3.700 mm (±1.153), then declined during the 2nd hour at 2.90 mm (±0.400) and gradually decreased to 2.133 mm (±0.635), by the 4th and 5th hour it measured to 1.60 mm (± 0.458) and 1.60 mm (±0.346), and its lowest at 1.40 mm (±0.17) on the 6th hour. This suggests a time-dependent reduction in paw swelling over the observational period.

Table 18. One Way ANOVA results compare between the positive control, negative control, and the *S. alba* ethanolic seed extract (low, mid, and high dose of the LD50).

One Way ANOVA Result	**f-value**	**p-value**	**Remarks**
Initial	0.462629	0.76190	Nonsignificant
0 hr	2.28846	0.13148	Nonsignificant

1st hr	1.50412	0.27308	Nonsignificant
2nd hr	17.45248	0.00017	Significant
3rd hr	11.91582	0.00081	Significant
4th hr	11.48606	0.00093	Significant
5th hr	38.61111	0	Significant
6th hr	55.60976	0	Significant

Note: If f-value <0.05 and p-value <0.01 it is significant

To evaluate the statistical significance of differences in paw volume among the treatment groups, the researchers used One Way ANOVA at a f-value <0.05 level of significance. As it states above in Table 18, the One Way ANOVA result reveals if there is a significant difference between the Negative Control, Positive Control, and the varying doses of *S. alba* ethanolic seed extract (Low, Mid, High) the specific time frame.

At the initial paw measurement the f = 0.462629, p = 0.76190, which means there were no significant differences. During the early phase of inflammation, the treatments including the extract and controls have not yet exerted distinguishable anti-inflammatory effects. In 0 hour, the paw measurement the f = 2.28846, p = 0.13148, means there is also no significant difference and also in the 1st hour the f- value 1.50412, p-value 0.27308. By the 2nd hour, f-value 17.45248, p-value 0.00017, this marks the onset of anti-inflammatory activity, where the extract begins to significantly reduce edema compared to the control groups. In the 3rd hour the f-value 11.91582, p-value 0.00081, the F-value remains high, indicating continued large differences among groups and progressive increase in the anti-inflammatory effect of the treatments. While in the 4th hour, f-value 11.48606 and p-value 0.00093, shows the F-value is still substantially high, indicating that the treatments continue to differ significantly in their effects. By the 5th hour the f-value 38.61111 and p-value 0.00000, shows a large difference. This suggests that the treatments, particularly the extract and positive control, are showing strong anti-inflammatory effects, clearly distinguishing them from the negative control. And, lastly, 6th hour, f-value 55.60976, p-value 0.00000, this reflects the maximum anti-inflammatory effect, where treated groups show near-complete resolution of inflammation compared to the negative control.The anti-inflammatory activity of *S. alba* ethanolic seed extract becomes statistically significant starting at the 2nd hour and continues to increase over time, as evidenced by the rising F-values and decreasing p-values. This confirms that the extract exhibits a delayed but strong anti-inflammatory effect, particularly during the later stages of inflammation.

Table 19. Post Hoc Tukey HSD test result of Initial hour comparing the control group (Positive and Negative) and the treatments of *S. alba* ethanolic seed extract (low, mid, and high dose of the LD50)

Post Hoc Tukey HSD Test	**P-Value**	
Positive-Negative	0.8643	Nonsignificant
Positive-Low	0.8643	Nonsignificant

Positive-Mid	0.8643	Nonsignificant
Positive- High	0.9996	Nonsignificant
Negative-Low	1.0000	Nonsignificant
Negative-Mid	1.0000	Nonsignificant
Negative-High	0.9329	Nonsignificant
High-Low	0.9329	Nonsignificant
High-Mid	0.9329	Nonsignificant
Low-Mid	1.0000	Nonsignificant

Note: If p-value <0.05 and p-value <0.01 it is significant

Following the result of the One Way ANOVA test for initial paw volume (p=0.76190), the post hoc test was performed to determine whether there are significant pairwise differences between the control groups (positive and negative) and the treatment groups (low, mid, and high doses of *S. alba* ethanolic seed extract) during the initial hour. The Positive vs Negative = 0.8643, Positive vs Low = 0.8643, Positive vs Mid = 0.8643, Positive vs High = 0.9996, Negative vs Low = 1.0000, Negative vs Mid = 1.0000, Negative vs High = 0.9329, High vs Low = 0.9329, High vs Mid = 0.9329, Low vs Mid = 1.0000. Since all computed p-values are greater than 0.05, this indicates that there are no statistically significant differences between any pair of groups at the initial hour.The positive control, negative control, and all extract doses (low, mid, and high) exhibit similar mean paw edema values at baseline.

Table 20. Post Hoc Tukey HSD test result of 0 hour comparing the control group (Positive and Negative) and the treatments of *S. alba* ethanolic seed extract (low, mid, and high dose of the LD50)

Post Hoc Tukey HSD Test	**P-Value**	**Remarks**
Positive-Negative	1.0000	Nonsignificant
Positive-Low	0.1679	Nonsignificant
Positive-Mid	1.0000	Nonsignificant
Positive-High	0.9837	Nonsignificant
Negative-Low	0.1901	Nonsignificant
Negative-Mid	1.0000	Nonsignificant
Negative-High	0.9917	Nonsignificant

High-Low	0.3410	Nonsignificant
High-Mid	0.9837	Nonsignificant
Low-Mid	0.1679	Nonsignificant

Note: If p-value <0.05 and p-value <0.01 it is significant

Following the result of the One Way ANOVA test for initial paw volume (p=0.13148), the post hoc test was performed to determine whether there are significant pairwise differences between the control groups (positive and negative) and the treatment groups (low, mid, and high doses of *S. alba* ethanolic seed extract) during the 0 hour. Specifically, the comparison between the positive and negative control (p = 1.0000) shows that both control groups exhibited comparable levels of paw edema immediately after induction, meaning the inflammatory response was uniformly established. The positive control versus low dose (p = 0.1679) and positive control versus mid dose (p = 1.0000) also showed no significant differences, indicating that the extract at these concentrations has not yet produced any distinguishable anti-inflammatory effect at this early stage. Similarly, the positive control versus high dose (p = 0.9837) suggests that even the highest concentration of the extract has not yet shown a measurable effect compared to the standard drug. The negative control versus low dose (p = 0.1901), negative control versus mid dose (p = 1.0000), and negative control versus high dose (p = 0.9917) likewise revealed no significant differences, confirming that the extract-treated groups are still comparable to the untreated inflamed group at this time point. Furthermore, comparisons among the extract doses (high vs low: p = 0.3410, high vs mid: p = 0.9837, low vs mid: p = 0.1679) also showed no significant differences, indicating that there is no dose-dependent effect yet observed at 0 hour. At 0 hour, the post hoc test showed no significant differences among all groups since all p-values are greater than 0.05. This indicates that the inflammation induced was consistent across all groups and that the treatments have not yet produced any observable anti-inflammatory effect at this early stage.

Table 21. Post Hoc Tukey HSD test result of 2nd our comparing the control group (Positive and Negative) and the treatments of *S. alba* ethanolic seed extract (low, mid, and high dose of the LD50)

Post Hoc Tukey HSD Test	**P-Values**	**Remarks**
Positive-Negative	0.6854	Nonsignificant
Positive-Low	0.0854	Nonsignificant
Positive-Mid	0.0007	Significant
Positive-High	0.0531	Nonsignificant
Negative-Low	0.0111	Significant
Negative-Mid	0.0002	Significant

Negative=High	0.0070	Significant
High-Low	0.9978	Nonsignificant
High-Mid	0.0850	Nonsignificant
Low-Mid	0.0531	Nonsignificant

Note: If p-value<0.05 and p-value 0.01 it is significant

Following the result of the ANOVA test for initial paw volume (p=0.00017), the post hoc test was performed to determine whether there are significant pairwise differences between the control groups (positive and negative) and the treatment groups (low, mid, and high doses of *S. alba* ethanolic seed extract) during the 2nd hour. The comparison between the positive and negative control (p = 0.3239) showed no significant difference, indicating that although inflammation is decreasing, both groups still exhibit relatively comparable edema levels at this stage. The comparison between the positive control and low dose (p = 0.0652) was not significant, suggesting that the low dose extract produces a similar effect to the standard drug at this time point. However, the comparison between the positive control and mid dose (p = 0.0158) showed a statistically significant difference, indicating that the mid dose exhibits a different level of anti-inflammatory activity compared to the positive control. In contrast, the positive control versus high dose (p = 0.3638) showed no significant difference, suggesting that the high dose effect is comparable to the standard drug. When comparing the negative control with the extract-treated groups, significant differences were observed: negative versus low dose (p = 0.0031), negative versus mid dose (p = 0.0009), and negative versus high dose (p = 0.0182), all indicating that the extract significantly reduces inflammation compared to the untreated group. Meanwhile, comparisons among the extract doses themselves, high versus low (p = 0.7556), high versus mid (p = 0.2872), and low versus mid (p = 0.8839) were not significant, suggesting that despite differences in magnitude, the effects of the different concentrations are not statistically distinguishable at this time point. These findings indicate that by the 3rd hour, the *S. alba* extract demonstrates significant anti-inflammatory activity compared to the negative control and showed comparable effects to the positive control in most cases, although the mid dose may exhibit a distinct response.

Table 22. Post Hoc Tukey HSD test result of 3rd hour comparing the control group (Positive and Negative) and the treatments of *S. alba* ethanolic seed extract (low, mid, and high dose of the LD50)

Post Hoc Tukey HSD Test	**P-Value**	**Remarks**
Positive-Negative	0.3239	Nonsignificant
Positive-Low	0.0652	Nonsignificant
Positive-Mid	0.0158	Significant
Positive-High	0.3638	Nonsignificant

Negative-Low	0.0031	Significant
Negative-Mid	0.0009	Significant
Negative-High	0.0182	Significant
High-Low	0.7556	Nonsignificant
High-Mid	0.2872	Nonsignificant
Low-Mid	0.8839	Nonsignificant

Note: If p-value <0.05 and p-value <0.01 it is significant

Following the result of the ANOVA test for initial paw volume (p=0.00081), the post hoc test was performed to determine whether there are significant pairwise differences between the control groups (positive and negative) and the treatment groups (low, mid, and high doses of *S. alba* ethanolic seed extract) during the 3rd hour. Positive and negative control (p = 0.3239) showed no significant difference, indicating that both groups have similar levels of edema at this time point. Likewise, the comparison between the positive control and low dose (p = 0.0652) was not significant, suggesting that the low dose produces an effect comparable to the standard drug. The positive control versus mid dose (p = 0.0158) showed a significant difference, indicating that the mid dose has a distinct level of anti-inflammatory activity compared to the positive control. The comparison between the positive control and high dose (p = 0.3638) remained not significant, implying that the high dose exhibits a similar effect to the standard treatment. Comparisons between the negative control and the extract-treated groups low (p = 0.0031), mid (p = 0.0009), and high dose (p = 0.0182) were statistically significant, demonstrating that all extract concentrations effectively reduced inflammation relative to the untreated group. On the other hand, comparisons among the extract doses themselves high versus low (p = 0.7556), high versus mid (p = 0.2872), and low versus mid (p = 0.8839) were not significant, indicating that the differences in their effects are not statistically meaningful. Overall, these findings suggest that by the 3rd hour, the *S. alba* extract has begun to exhibit notable anti-inflammatory activity.

Table 23. Post Hoc Tukey HSD test result of 4th hour comparing the control group (Positive and Negative) and the treatments of *S. alba* ethanolic seed extract (low, mid, and high dose of the LD50)

Post Hoc Tukey HSD Test	**P-Value**	**Remarks**
Positive-Negative	0.0801	Nonsignificant
Positive-Low	0.0924	Nonsignificant
Positive-Mid	0.1226	Nonsignificant
Positive-High	0.8776	Nonsignificant
Negative-Low	0.0011	Significant

Negative-Mid	0.0015	Significant
Negative-High	0.0189	Significant
High-Low	0.3489	Nonsignificant
High-Mid	0.4372	Nonsignificant
Low-Mid	0.9997	Nonsignificant

Note: If p-value <0.05 and p-value <0.01 it is significant

Following the result of the ANOVA test for initial paw volume (p=0.00093), the post hoc test was performed to determine whether there are significant pairwise differences between the control groups (positive and negative) and the treatment groups (low, mid, and high doses of *S. alba* ethanolic seed extract) during the 4th hour. The comparison between the positive and negative control (p = 0.0801) showed no significant difference, suggesting that although inflammation is decreasing, the difference between these two groups is not yet statistically distinct. Similarly, the comparisons between the positive control and the extract-treated groups low dose (p = 0.0924), mid dose (p = 0.1226), and high dose (p = 0.8776) were all not significant, indicating that the anti-inflammatory effects of the extract at all concentrations are comparable to the standard drug at this stage. In contrast,comparisons between the negative control and the extract-treated groups low dose (p = 0.0011), mid dose (p = 0.0015), and high dose (p = 0.0189) were statistically significant, demonstrating that all extract doses effectively reduce inflammation compared to the untreated group. Meanwhile, comparisons among the extract doses themselves high versus low (p = 0.3489), high versus mid (p = 0.4372), and low versus mid (p = 0.9997) were not significant, indicating that the differences among concentrations are not statistically meaningful.

Table 24. Post Hoc Tukey HSD test result of 5th hour comparing the control group (Positive and Negative) and the treatments of *S. alba* ethanolic seed extract (low, mid, and high dose of the LD50)

Post Hoc Tukey HSD Test	P-Value	Remarks
Positive-Negative	<0.0001	Significant
Positive-Low	0.5898	Nonsignificant
Positive-Mid	0.1862	Nonsignificant
Positive-High	1.0000	Nonsignificant
Negative-Low	<0.0001	Significant
Negative-Mid	<0.0001	Significant
Negative-High	<0.0001	Significant

High-Low	0.5898	Nonsignificant
High-Mid	0.1862	Nonsignificant
Low-Mid	0.8783	Nonsignificant

Note: If p-value <0.05 and p-value <0.01 it is significant

Following the result of the ANOVA test for initial paw volume (p=0.0), the post hoc test was performed to determine whether there are significant pairwise differences between the control groups (positive and negative) and the treatment groups (low, mid, and high doses of *S. alba* ethanolic seed extract) during the 5th hour. The positive and negative control ($p < 0.0001$) was highly significant, indicating that the standard drug has significantly reduced inflammation compared to the untreated group. Comparisons between the positive control and the extract-treated groups low dose ($p = 0.5898$), mid dose ($p = 0.1862$), and high dose ($p = 1.0000$) were not significant. All comparisons between the negative control and the extract-treated groups (all $p < 0.0001$) were highly significant, confirming that the extract effectively reduces inflammation relative to the untreated group. On the other hand, comparisons among the extract doses themselves high versus low ($p = 0.5898$), high versus mid ($p = 0.1862$), and low versus mid ($p = 0.8783$) remained not significant, indicating no meaningful difference in effect among the different concentrations.

Table 25. Post Hoc Tukey HSD test result of 6th hour comparing the control group (Positive and Negative) and the treatments of *S. alba* ethanolic seed extract (low, mid, and high dose of the LD50)

Post Hoc Tukey HSD Test	**P-Value**	**Remarks**
Positive-Negative	<0.0001	Significant
Positive-Low	0.8550	Nonsignificant
Positive-Mid	0.5950	Nonsignificant
Positive-High	0.8550	Nonsignificant
Negative-Low	<0.0001	Significant
Negative-Mid	<0.0001	Significant
Negative-High	<0.0001	Significant
High-Low	1.0000	Nonsignificant
High-Mid	0.9862	Nonsignificant
Low-Mid	0.9862	Nonsignificant

Note: If p-value <0.05 and p-value 0.01 it is significant

Following the result of the ANOVA test for initial paw volume (p=0.0), the post hoc test was performed to determine whether there are significant pairwise differences between the control groups (positive and negative) and the treatment groups (low, mid, and high doses of *S. alba* ethanolic seed extract) during the 6th hour. The positive and negative control ($p < 0.0001$) shows a highly significant difference, confirming that the standard drug effectively reduced inflammation compared to the untreated group. In contrast, the comparisons between the positive control and the extract-treated groups low dose ($p = 0.8550$), mid dose ($p = 0.5950$), and high dose ($p = 0.8550$) revealed no significant differences, suggesting that all concentrations of the *S. alba* extract have achieved a level of anti-inflammatory effect comparable to the standard drug by this time point. Negative control and the extract-treated groups were highly significant ($p < 0.0001$), indicating that the extract consistently and effectively reduced paw edema relative to the untreated condition. This further supports the strong anti-inflammatory activity of the extract during the late phase of inflammation. On the other hand, the absence of significant differences among the extract doses themselves—high versus low ($p = 1.0000$), high versus mid ($p = 0.9862$), and low versus mid ($p = 0.9862$) suggests that increasing the concentration does not result in a significantly greater effect at this stage.

Table 26. Linear regression result of the anti-inflammatory potential of *S. alba* ethanolic seed extract is concentration dependent (low, mid, and high of median lethal dose)

Time	Intercept	Estimate (Slope)	P-Value	Direction
Initial	1.177778	0.006667	0.3686720	Increase
0 hr	2.644444	0.383333	0.1577543	Increase
1st hr	4.166667	-0.183333	0.5878978	Decrease
2nd hr	2.611111	-0.050000	0.8657495	Decrease
3rd hr	1.733333	0.216667	0.3909384	Increase
4th hr	1.155556	0.350000	0.1013444	Increase
5th hr	1.366667	0.133333	0.2989503	Increase
6th hr	1.377778	-5.665583e-17	1.0000000	Decrease

Note: If p-value <0.05 and p-value<0.01 it is significant

Linear regression was applied to evaluate whether the anti-inflammatory of S. alba ethanolic seed extract is concentration-dependent. The table above shows no significant linear relationship (p-value > 0.05) and inconsistent slopes across all time points indicating that the assumption of linearity is not met. This also suggests that the relationship between level of concentration and anti-inflammatory effect is not concentration-dependent within the tested range. Although the extract exhibited anti-inflammatory activity over time, increasing the concentration from low to high doses did not produce a consistent or statistically significant enhancement of effect.

This supports literature suggesting that compounds present in plants such as tannins, flavonoids and phenols can compete with synthetic NSAIDs in anti-inflammatory action as mentioned by (Sundari et al. 2022) and (Pavithran and Sujatha 2022).

4.7 Discussion

The ethanolic seed extract of *Sonneratia alba* was evaluated for its potential anti-inflammatory activity through phytochemical screening, oral toxicity testing, and carrageenan induced paw edema assay in mice. The extraction process produced a dark brown extract with a viscous consistency, suggesting a successful concentration of bioactive compounds present in the seeds. The average percentage yield obtained in the extraction was 64.507%, indicating that the seeds of *S. alba* contained a considerable amount of ethanol-soluble phytochemical constituents. The use of 95% ethanol as the extraction solvent may have contributed to the high extraction efficiency as ethanol was capable of dissolving both polar and nonpolar compounds. Variations observed among the three extraction trials may have resulted from differences in solvent filtration, extraction efficiency, and phytochemical distribution within the samples.

Phytochemical screening confirmed the presence of bioactive compounds such as alkaloids, tannins, steroids, saponins, flavonoids, phenols and terpenoids in the ethanolic seed extract of *S. alba*. The presence of the secondary metabolites suggests that the plant possesses significant pharmacological potential. Flavonoids and phenolic compounds are widely recognized for their anti-inflammatory activities as they inhibit the release of inflammatory mediators. Tannins are also known to stabilize biological membranes and reduce oxidative stress, saponins and terpenoids may suppress inflammatory pathways and minimize tissue damage caused by free radicals. Alkaloids and steroids may further contribute to the anti-inflammatory effect due to their analgesic-like activities. These findings support previous studies reporting that *S. alba* contains several biologically active compounds that are responsible for its therapeutic effect. The phytochemical profile observed in this study therefore provides scientific evidence supporting the potential anti-inflammatory activity of *S. Alba* ethanolic seed extract.

Following the OECD 423 Acute Oral Toxicity Class Method, the acute oral toxicity test was conducted to evaluate the safety profile of the extract. No mortality or abnormal behavioural changes were observed in mice administered with the doses ranging from 5mg/kg to 2000mg/kg during the first 24 hours after administration. While mice that were administered with 5000mg/kg did not exhibit an immediate toxic reaction. However, one mortality was observed on the seventh day after the administration of 5000mg/kg dose. Gross necropsy findings revealed an enlarged or swollen intestines in the affected mouse, while no other visible abnormalities were observed in the other organs. Since only one out of three mice died at the limit dose and no consistent toxic manifestation were observed in the remaining animals, the medial lethal dose (LD_{50}) of the extract was estimated to be greater than 5000mg/kg. According to OECD classification, substances with LD_{50} values greater than 5000mg/kg are generally considered to possess low acute toxicity effects observed in this study indicating that the ethanolic seed extract of *S. alba* has a favorable safety profile at therapeutic dose levels.

The anti-inflammatory of the *S. alba* was evaluated using the carrageenan-induced paw edema in mice. Carrageenan causes inflammation and swelling in the paw, making it a commonly used method for testing anti-inflammatory agents. The extract showed the ability to reduce paw inflammation at different dose levels, indicating its potential anti-inflammatory effect. The positive control group was treated with diclofenac sodium and showed a strong inhibition of inflammation, but the extract-treated groups also

demonstrated a noticeable reduction in paw edema compared to the negative control group which was treated with normal saline solution.

The anti-inflammatory effect observed in this study may be linked to the phytochemical present in the extract particularly flavonoids, phenols, tannins, and terpenoids, which are known to help inhibit inflammatory reactions. Similar studies on *S. alba* also reported that the plant possesses antioxidant and anti-inflammatory properties due to its bioactive compounds. The differences observed among the dose groups may be due to the varying concentration of active compounds present in each dose levels (low, mid, high).

In statistical analysis it showed that there were significant differences in the anti-inflammatory effects among the treatment groups. In one- way ANOVA the anti-inflammatory activity of the extract becomes statistically significant at the second hour with a f-value of 17.45248 and a p-value of 0.00017. Since the p-value was lower than 0.05, thus the null hypothesis was rejected, indicating that the treatments produced different effects in reducing paw edema.

The results further showed that the anti-inflammatory activity of the extract was time-dependent, as the reduction in paw swelling or inflammation became more noticeable during the later hours of observation. This suggests that the ethanolic seed extract gradually exerted its effect as the inflammatory response progressed. The increase in anti-inflammatory activity over time may be associated with the second phase of carrageenan-induced inflammation, which involves the release of prostaglandins and other inflammatory mediators that the phytochemicals in the extract may help suppress.

The post hoc Tukey's test conducted using the 5th-hour data further identified the specific differences among treatment groups. The comparison between the negative control and diclofenac sodium showed a highly significant difference with a p-value of <0.0001, confirming the strong anti-inflammatory activity of diclofenac sodium. The middle dose group also showed a significant reduction in inflammation compared to the negative control group, with a p-value of <0.001, while the high dose group demonstrated the same effect with a p-value of <0.0001. However, there was no significant difference between the middle and high dose groups, with a p-value of 0.1862, indicating comparable anti-inflammatory effects at these concentrations.

The greater reduction in paw edema observed in the higher dose groups and during the later hours suggests that the extract may exhibit both dose-dependent and time-dependent anti-inflammatory activity. This effect may be attributed to the presence of flavonoids, phenols, tannins, and terpenoids identified during phytochemical screening, which are known to inhibit inflammatory mediators responsible for swelling and tissue inflammation. Overall, the findings suggest that the ethanolic seed extract of *Sonneratia alba* possesses significant anti-inflammatory activity, particularly at higher doses and longer durations of observation.

CHAPTER 5
CONCLUSION, AND DIRECTIONS FOR FUTURE RESEARCHERS

The study aimed to evaluate the anti-inflammatory potential, phytochemical constituents, and safety profile of the 95% ethanolic seed extract of *S. alba* using an acute edema model. Through a series of laboratory analyses and *in vivo* tests, the research generated valuable data supporting its potential as a natural therapeutic agent. The following summarizes the key findings derived from the experimental procedures:

5.1 SUMMARY OF FINDINGS

1. The 95% ethanolic seed extract of *Sonneratia alba* produced a mean percentage yield of 64.507% from triplicate analysis of 10-gram dried seed samples. Trial 1 yielded 5.385 g or 53.85%, Trial 2 yielded 7.569 g or 75.69%, and Trial 3 yielded 6.398 g or 63.98%. These results indicate that the seeds of *S. alba* contain a considerable amount of ethanol-soluble constituents.
2. Phytochemical screening confirmed the presence of alkaloids, flavonoids, phenols, saponins, steroids, tannins, and terpenoids in the ethanolic seed extract of *S. alba*. Among these constituents, alkaloids, flavonoids, phenols, saponins, steroids, and tannins were detected in higher concentration (+++), while terpenoids were present in moderate amounts (++). This suggests that the extract contains bioactive compounds that may contribute to its pharmacological activity, particularly its anti-inflammatory potential.
3. Acute oral toxicity testing showed that no abnormal behavior or mortality was observed in mice administered 5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg within the first 24 hours. At 5000 mg/kg, no immediate mortality was noted during the first 24 hours; however, one mouse died on the seventh day. Based on these findings, the lethal dose of the extract was estimated at 5000 mg/kg. The anti-inflammatory dose levels used in the experiment were 50 mg/kg for the low dose, 300 mg/kg for the mid dose, and 2000 mg/kg for the high dose.
4. The different concentrations of *S. alba* ethanolic seed extract showed anti-inflammatory activity in the carrageenan-induced paw edema model. The extract reduced paw edema compared with the negative control (Normal Saline Solution), particularly at later time points. All treatment groups demonstrated the ability to reduce inflammation in mice.
5. Comparison with the positive control (diclofenac sodium) showed that the mid dose of *S. alba* extract produced the most pronounced anti-inflammatory effect and was reported to be comparable to diclofenac sodium. A statistically significant difference was observed at the 3rd hour ($p = 0.0006$), indicating a strong anti-inflammatory activity of the extract under the conditions of the study.
6. Analysis of variance revealed no significant differences among groups during the early time points, but significant differences were observed from the 2nd hour to the 6th hour. The post hoc Tukey HSD test further showed that the mid dose produced the strongest therapeutic effect compared with the negative control and had a response comparable to the positive control at later time points.
7. Linear regression analysis showed no linear relationship between dose and effect. Although the extract demonstrated anti-inflammatory activity, its response was found to be time-dependent rather than dose dependent. Thus, the anti-inflammatory activity of the ethanolic seed extract of *S. alba* was not concentration-dependent within the tested dose range.

5.2 CONCLUSION

The extract of *S. alba* demonstrated potential anti-inflammatory activity based on the results obtained; it exhibited a significant reduction in paw edema in the acute edema model. An *in vivo* experiment produced useful evidence supporting its potential as a natural medicinal agent. The findings showed that the extract had a time-dependent anti-inflammatory effect, with the mid dosage being the most effective, producing the largest reduction in paw edema and exhibiting activity nearly comparable to Diclofenac sodium. A statistically significant difference was observed during the 5th to 6th hour between the positive and negative control groups, as well as between the extract-treated groups and the negative control, further supporting the efficacy of the extract at later time intervals. Phytochemical analysis confirmed the

presence of various bioactive constituents, including alkaloids, flavonoids, phenols, saponins, steroids, and tannins which are primarily responsible for the observed anti-inflammatory effects. However, acute toxicity studies found that the extract is highly toxic at 5000 mg/kg, indicating a lethal dosage. Based on these results, *S. alba* indicates potential as a natural source of anti-inflammatory compounds, indicating its potential application in the development of plant-based medicinal alternatives, while emphasizing the importance of careful dose measurement.

5.3 DIRECTION FOR FUTURE RESEARCHERS

1. Future researchers may use a larger number of experimental animals per group to improve the reliability and consistency of the results.
2. It is recommended to test additional doses such as 25%, 50%/ and 75% of the LD50 to better identify the most effective anti-inflammatory dose of the extract.
3. Future studies may include more precise and consistent observation time points to clearly monitor the onset and duration of the anti-inflammatory effect.
4. Quantitative phytochemical analysis may also be conducted to determine the amount of each phytochemical constituent present in the extract.
5. Further toxicity studies, such as subacute toxicity testing and long-term, may be done to evaluate the safety of the extract after repeated administration.
6. Other plant parts of *Sonneratia alba*, such as the leaves, bark, or fruit, may also be investigated and compared with the seed extract in terms of anti-inflammatory activity.
7. Future researchers may use other extraction methods or solvent concentrations to determine whether a better yield and stronger anti-inflammatory effect can be obtained.
8. Additional studies may be conducted using other simple inflammation models to further support the anti-inflammatory potential of *S. alba* extract.
9. To further study and identify the bioactive components present in the ethanolic seed extract.

REFFERENCE

Journals

1. Alam P, Alqahtani AS, Husain FM, Rehman MT, Alajmi MF, Noman OM, et al. 2012. In vitro antimicrobial activity of methanol and ethyl acetate extracts of *Sonneratia alba*. *Saudi Pharm J.* 28(11):1383–1391. doi:10.1016/S2221-1691(12)60069-0.

2. Andriani Y, Hanifah W, Kholieqoh AH, Abdul Majid FA, Hermansyah H, Amir H, Muhammad TST. 2023. Antibacterial activity of hexane and methanol fractions of some selected plants against Klebsiella pneumoniae. J Adv Pharm Technol Res. 14(3):220-225. doi: 10.4103/JAPTR.JAPTR_183_23.
3. Antiplasmodial Activity of Ethanol Extract of Sonneratia alba Leaves. 2024. Tropical Journal of Natural Product Research. 8(4). doi: https://doi.org/10.26538/tjnpr/v8i4.19
4. Asad S, Nesa L, Deepa KN, Sohel KM. 2017. Analgesic, anti-inflammatory and CNS depressant activities of methanolic extract of Sonneratia alba leaves in mice. Natl Prod Chem Res. 5:277–84.
5. Asih, I.A.R.A., Ngongo, S., Manuaba, I.B.P. (2025). Anti-inflammatory activities of chitosan-TPP nanoformulated Wrightia pubescens R.Br leaf extract in carrageenan-induced paw edema of Wistar rats. International Journal of Design & Nature and Ecodynamics, Vol. 20, No. 5, pp. 1131-1137. https://doi.org/10.18280/ijdne.200517

6. Banaszak M, Dobrzyńska M, Kawka A, Górna I, Woźniak D, Przysławski J, Drzymała-Czyż S. 2024. Role of Omega-3 fatty acids eicosapentaenoic (EPA) and docosahexaenoic (DHA) as modulatory and anti-inflammatory agents in noncommunicable diet-related diseases - Reports from the last 10 years. Clin Nutr ESPEN. 63:240-258. doi: 10.1016/j.clnesp.2024.06.053.
7. Bindu S, Mazumder S, Bandyopadhyay U. 2020. Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: a current perspective. Biochem Pharmacol. 180:114147. doi:10.1016/j.bcp.2020.114147
8. Biswas P, Biswas SR. (pdf) mangrove forests: Ecology, management, and threats. Mangrove Forests: Ecology, Management, and Threats. [accessed 2025 Aug 15]. https://www.researchgate.net/publication/347141801_Mangrove_Forests_Ecology_Management_and_Threats
9. Boarescu I, Pop RM, Boarescu P-M, Bocșan IC, Gheban D, Râjnoveanu R-M, Râjnoveanu A, Bulboacă AE, Buzoianu AD, Bolboacă SD. 2022 Oct 3. Anti-inflammatory and analgesic effects of curcumin nanoparticles associated with diclofenac sodium in experimental acute inflammation. MDPI. https://www.mdpi.com/1422-0067/23/19/11737
10. Budi SB, Dwi ST, Hardoko. 2019. Phytochemicals and identification of antioxidant compounds from ethanol extract of Sonneratia alba leaves and bark. RJOAS. 11(95):190–196. doi:10.18551/rjoas.2019-11.26.
11. Buljan, Ivan. 2023. "Research Procedures." In *A Guide to Responsible Research*, edited by Ana Marušić, 31–47. Cham: Springer. https://doi.org/10.1007/978-3-031-22412-6_3
12. Cabassi A, Tedeschi S, Perlini S, Verzicco I, Volpi R, Gonzi G, Del Canale S. 2020. Non-steroidal anti-inflammatory drug effects on renal and cardiovascular function: from physiology to clinical practice. *Eur J Prev Cardiol.* 27(8):850–867. doi:10.1177/2047487319848105
13. Cabrera EC, de Guzman F, Santiago LA, Pocsidio GL, Abad SC, Lopez J, de Castro-Bernas G. 2021. Attenuation of carrageenan-induced hind paw edema and mechanical hyperalgesia by Philippine stingless bee (Tetragonula biroi) propolis in mice. *Experimental Animals* [Internet]. 70(2):220–228 [cited 2025 Sep 25]. Available from: https://www.jstage.jst.go.jp/article/expanim/advpub/0/advpub_20-0118/_article/-char/en
14. Casiple JGM, Amerol SA, Montebon JKA, Mangontra JR, Garay JGL. 2025 Jul 25. Investigation of the anti-inflammatory activity of Barringtonia asiatica (Lecythidaceae) ethanolic leaf extract in albino mice. Int J Innov Sci Res Technol. 1952-1956. doi:10.38124/iiisrt/25jul1121. Available from: https://eprint.innovativepublication.org/id/eprint/1972/1/
15. Chaniad P, Phuwajaroanpong A, Plirat W, Techarang T, Chukaew A, Punsawad C. 2022. In vivo assessment of the antimalarial activity and acute oral toxicity of an ethanolic seed extract of *Spondias pinnata* (L.f.) Kurz. *BMC Complement Med Ther.* 22(1):72. https://doi.org/10.1186/s12906-022-03546-9
16. Chen JT, Ostermann M. 2022. Review of Anti-inflammatory and Antiviral Therapeutics for Hospitalized Patients Infected with Severe Acute Respiratory Syndrome Coronavirus 2. Crit Care Clin. 38(3):587-600. doi: 10.1016/j.ccc.2022.02.002.
17. Di Rosa M, Giroud JP, Willoughby DA. 1971. Studies of the Mediators of the Acute Inflammatory Response Induced in Rats in Different Sites by Carrageenan and Turpentine. J Pathol. 104:15-29. doi:10.1002/path.1711040103

18. Dolanbay T, Makav M, Gul HF, Karakurt E. 2021. The effect of diclofenac sodium intoxication on the cardiovascular system in rats. Am J Emerg Med. 46:560-566. doi:10.1016/j.ajem.2020.11.022.
19. Dotulong V, Montolalu LADY, Wonggo D, Sanger G, Damongilala L, Syahputra RA. 2024. Boiling water extraction of mangrove Sonneratia alba fruit as an antioxidant functional food: combined in vitro and pharmacoinformatics studies. Nutr Clín Diet Hosp. 44(4):295–313. doi:10.12873/444.
20. Feng X, Xu S, Li J, Yang Y, Chen Q, Lyu H, Zhong C, He Z, Shi S. 2020. Molecular adaptation to salinity fluctuation in tropical intertidal environments of a mangrove tree Sonneratia alba. BMC Plant Biol. 20(1). https://doi.org/10.1186/s12870-020-02395-3
21. Galge A, Thange T. 2023. A review on diclofenac sodium. Int Res J Mod Eng Technol Sci. 5(4):5029–5034. [accessed 2025 Aug 27]. https://www.irjmets.com
22. Gazali N, Rosdiana A, Sari SP, Anjani G, Wahyuni DS. 2020. Qualitative and quantitative phytochemical differences among solvent extracts of *Sonneratia alba*. *J Pengolahan Hasil Perikanan Indones*. 23(2):345–354. doi:10.17844/jphpi.v23i2.31684.
23. Haq I, Hossain AS, Moneruzzaman M, Amir K, Merican F, Faruq G, et al. 2014. Antioxidant and antibacterial activities of different extracts and fractions of a mangrove plant Sonneratia alba. Int J Agric Biol. 16(4):707–14.
24. Iddir M, Brito A, Dingeo G, Fernandez Del Campo SS, Samouda H, La Frano MR, Bohn T. 2020. Strengthening the Immune System and Reducing Inflammation and Oxidative Stress through Diet and Nutrition: Considerations during the COVID-19 Crisis. Nutrients. 12(6):1562. https://doi.org/10.3390/nu12061562
25. Kim KH, Im H-W, Karmacharya MB, Kim S, Min B-H, Park SR, Choi BH. Low-intensity ultrasound attenuates paw edema formation and decreases vascular permeability induced by carrageenan injection in rats - journal of inflammation. BioMed Central. 2020 Feb 13 [accessed 2025 Aug 27]. https://journal-inflammation.biomedcentral.com/articles/10.1186/s12950-020-0235-x
26. Kuang YY, Gao X, Niu YJ, Shi XL, Zhou W. Polymorphic Characterization, Pharmacokinetics, and Anti-Inflammatory Activity of Ginsenoside Compound K Polymorphs. Molecules. 2021 Apr 1;26(7):1983. doi: 10.3390/molecules26071983. PMID: 33915860; PMCID: PMC8037814.
27. Kuchar E, Karlikowska-Skwarnik M, Wawrzuta D. 2022. Anti-Inflammatory Therapy of Infections. Encyclopedia of Infection and Immunity. 791–7. doi: 10.1016/B978-0-12-818731-9.00181-6.
28. Kulkarnii CP, Manohar SM. A review of botany, phytochemistry, and pharmacology of the mangrove apple Sonneratia Alba J. Sm. Journal of Applied Pharmaceutical Science. 2024 May 5 [accessed 2025 Aug 15]. https://japsonline.com/abstract.php?article_id=4214&sts=2
29. Kumar A, Sharma S. 2024. Evaluation of anti-inflammatory activity of plant extracts: a comparative study. *LS Int J Life Sci*. 14(1):125-132.
30. Kundu P, Debnath SL, Devnath HS, Saha L, Sadhu SK. 2022. Analgesic, anti-inflammatory, antipyretic, and in silico measurements of Sonneratia caseolaris (L.) fruits from Sundarbans, Bangladesh. Biomed Res Int. 2022:1405821. doi:10.1155/2022/1405821.
31. Lee J-E, Jayakody JTM, Kim J-I, Jeong J-W, Choi K-M, Kim T-S, Seo C, Azimi I, Hyun J, Ryu B. The influence of solvent choice on the extraction of bioactive compounds from Asteraceae: A comparative review. MDPI. 2024 Oct 2 [accessed 2025 Aug 27]. https://www.mdpi.com/2304-8158/13/19/3151
32. Lim WY, Cheng YW, Lian LB, Chan EWC, Wong CW. 2021. Inhibitory effect of Malaysian coastal plants on banana, ginger and sweet potato polyphenol oxidase. J Food Sci Technol. 58(11):4178–84.

33. Li Y, Mai Y, Cao P, Wen X, Fan T, Wang X, Ruan G, Tang S, Ding C, Zhu Z. 2022. Relative efficacy and safety of anti-inflammatory biologic agents for osteoarthritis: a meta-analysis. J Clin Med. 11(14):3958. doi:10.3390/jcm11143958
34. Manohar SM. 2021. A review of the botany, phytochemistry and pharmacology of mangrove Lumnitzera racemosa Willd. Pharmacogn Rev. 15(30):107–16. doi:10.5530/phrev.2021.15.13
35. Manuhuttu D, Saimima NA. 2021. Potential of mangrove leaf extract (Sonneratia alba) as antibacterial. Biopendix. 7(2):71-79.
36. Mata R, Yao Y, Ding J, Zho T, Zhai Z, Gao C. 2021. The dynamic inflammatory tissue microenvironment: Signality and disease therapy by Biomaterials. Research (Washington, D.C.). https://pubmed.ncbi.nlm.nih.gov/33623917/
37. Milon MA, Muhit MA, Goshwami D, Masud MM, Begum B. 2012. Antioxidant, cytotoxic, and antimicrobial activity of Sonneratia alba bark. Int J Pharm Sci Res. 3(7):2233-7
38. Morada NJ, Metillo EB, Uy MM, Oclarit JM. 2011. Anti-diabetic polysaccharide from mangrove plant, Sonneratia alba Sm. In: Proc Int Conf Asia Agric Anim. Hong Kong, China. 13:197–200.
39. Muhaimin M, Latief M, Putri RD, Chaerunisaa AY, Aditama AY, Pravitasari NE, et al. 2019. Antiplasmodial activity of methanolic leaf extract of mangrove plants against Plasmodium berghei. Pharmacog J. 11(5):929–35. doi:10.5530/pj.2019.11.148
40. Muhaimin M, Latifah N, Chaerunisaa AY, Subarnas A, Susilawati Y, Hirzan R. 2024. Antiplasmodial activity of ethanol extract of Sonneratia alba leaves. Trop J Nat Prod Res. 8(4):6884–6890. doi:10.26538/tjnpr/v8i4.19
41. Musa WJA, Bialangi N, Situmeang B, Silaban S. 2019. Triterpenoid compound from methanol extract of mangrove leaves (Sonneratia alba) and anticholesterol activity test. J Pendidikan Kimia. 11:18–23
42. Narimani Zamanabadi S, Hemmati AA, Zalaghi R, Sohrabi M, Eslami Samarin M, Nejaddehbashi F, Kiashi F. 2025. Assessment of the anti-inflammatory effects of several native macrolichens on carrageenan-induced paw edema in rats. *Jundishapur Journal of Natural Pharmaceutical Products.* 20(2):e158567. doi:10.5812/jjnpp-158567.
43. Noureen S, Noreen S, Ghumman SA, Al-Hussain SA, Hameed H, Anwar-Ul-Haq M, Zaki MEA, Aslam S. 2023. Maximizing extraction yield of plant gum exudate using response surface methodology and artificial neural network: a pharmacological characterization. Sci Rep. 13:37847. [accessed 2025 Aug 27]. https://www.nature.com/articles/s41598-023-37847-x
44. Nunes CDR, Arantes MB, De Faria Pereira SM, Da Cruz LL, De Souza Passos M, De Moraes LP, Vieira IJC, De Oliveira DB. 2020. Plants as sources of anti-inflammatory agents. Molecules. 25(16):3726. doi:10.3390/molecules25163726.
45. OECD/OCDE 423 OECD GUIDELINE FOR TESTING OF CHEMICALS Acute Oral Toxicity - Acute Toxic Class Method. 2001. https://ntp.niehs.nih.gov/sites/default/files/iccvam/suppdocs/feddocs/oecd/oecd_gl423.pdf.
46. Oronsky B, Caroen S, Reid T. 2022 Nov 28. What exactly is inflammation (and what is it not?). MDPI. https://www.mdpi.com/1422-0067/23/23/14905
47. Otari SS, Patil YK, Patel SB, Lekhak MM, Rastogi A, Kumar V, Ghane SG. 2025. Secondary metabolites and biological activities of the mangrove apple (*Sonneratia alba*). In: Reference Series in Phytochemistry. p. 1–17. doi:10.1007/978-3-031-63920-3_19-1.
48. O'Brien WM. 1986. Adverse reactions to NSAIDs: diclofenac vs others. Am J Med. 80(4):70–80. doi:10.1016/0002-9343(86)90084-7

49. Patil KR, Mahajan UB, Unger BS, Goyal SN, Belemkar S, Surana SJ, Ojha S, Patil CR. 2019. Animal Models of Inflammation for Screening of Anti-inflammatory Drugs. Int J Mol Sci. 20(18). doi:10.3390/ijms20184367
50. Pavithran S, Sujatha PS. 2022. Evaluation of anti-inflammatory properties of ethanolic leaf, flower and seed extracts of Lagerstroemia speciosa (L.) Pers (Lythraceae) against carrageenan-induced acute inflammation in albino rats. J Adv Sci Res. 13(9):48-53. doi:10.55218/JASR.202213907.
51. Posadas I, Bucci M, Roviezzo F, Rossi A, Parente L, Sautebin L, Cirino G. 2023. Anti-inflammatory effect of Izalpinin in λ-carrageenan-induced paw edema in mice. Molecules [Internet]. 28(9):3722 [cited 2025 Sep 25]. Available from: https://www.mdpi.com/1420-3049/28/9/3722
52. Ramli ESM, et al. 2025. Lepisanthes alata attenuates carrageenan-induced paw edema in mice. Experimental Animals [Internet]. 74(2):123–131 [cited 2025 Sep 25]. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC12389709
53. Raut S, Anthapan PD. 2013. Studies on antimicrobial compound of extract of bark Sonneratia alba. Trend Biosci. 6(6):831-7.
54. Rizki A, Azmi W, Muhaimin M, Louisa M, Artika I, Siregar J. 2023. Pharmacological Activities of Sonneratia Alba Mangrove Plant: A Review. J Indones Soc Integr Chem. 15:128-138. doi:10.22437/jisic.v15i2.29274
55. Rossi J-F, Lu ZY, Massart C, Levon K. 2021. Dynamic immune/inflammation precision medicine: the good and the bad inflammation in infection and cancer. Front Immunol. 12:595722. doi: 10.3389/fimmu.2021.595722.
56. Saad S, Taher M, Susanti D, Qaralleh H, Awang AFIB. 2012. In vitro antimicrobial activity of mangrove plant Sonneratia alba. Asian Pac J Trop Biomed. 2(6):427-9. doi:10.1016/s2221-1691(12)60069-0
57. Sadeer NB, Zengin G, Mahomoodally MF. 2023. Biotechnological applications of mangrove plants. Crit Rev Biotechnol. 43:393-414. doi:10.1080/07388551.2022.2033682
58. Sahoo G, Mulla NSS, Ansari ZA, Mohandas C. 2012. Antibacterial activity of mangrove Leaf Extract against human pathogens. Indian J Pharm Sci. 74(4):348-51.
59. Semis HS, Gur C, Ileriturk M, Kaynar O, Kandemir FM. Investigation of the anti-inflammatory effects of caffeic acid phenethyl ester in a model of λ-Carrageenan-induced paw edema in rats. Hum Exp Toxicol. 2021 Dec;40(12_suppl):S721-S738. doi: 10.1177/09603271211054436. Epub 2021 Nov 18. PMID: 34789018.
60. Sewell F, Ragan I, Horgan G, Andrew D, Holmes T, Manou I, Müller BP, Rowan T, Schmitt BG, Corvaro M. 2024. New supporting data to guide acute oral toxicity studies. Regul Toxicol Pharmacol. 146:105517. doi:10.1016/j.yrtph.2023.105517
61. Sharma JN, Samud AM, Asmawi MZ. 2004. Comparison between plethysmometer and micrometer methods. Inflammopharmacology. 12(1):89-94. doi:10.1163/156856004773121400
62. Sharudin SZ. 2014. Anti-cholinergic and anti-microbial properties of Sonneratia alba (Perepat) [Undergraduate thesis]. Terengganu, Malaysia: Universiti Malaysia.
63. Shukla S, Srivastava S, Kumar M, Tiwari A, Mishra S, Ahmad M, et al. 2022. Effect of diclofenac and andrographolide combination on carrageenan-induced paw edema and hyperalgesia in rats. Journal of Experimental Pharmacology [Internet]. 14:293–303 [cited 2025 Sep 25]. Available from: https://journals.sagepub.com/doi/full/10.1177/15593258221103846

64. Siahaya VG, Moniharapon T, Mailoa MN, Leatemia JA. 2018. Potential of mangrove apples (Sonneratia alba) as a botanical insecticide. Mod Appl Sci. 12(1):1–8.
65. Singh TR, Ezhilarasan D. 2022. Lagerstroemia speciosa Pers. (Lythraceae) ethanolic leaves extract attenuates dapsone-induced liver inflammation in rats. Drug Chem Toxicol. 45(5):2361-2370. doi:10.1080/01480545.2021.1945079.
66. Suh SS, Hwang J, Park M, Park HS, Lee TK. 2014. Phenol content, antioxidant and tyrosinase inhibitory activity of mangrove plants in Micronesia. Asian Pac J Trop Med. 7(7):531–5.
67. Sumartini, Ratrinia PW, Hutabarat RF. 2022. The effect of mangrove types and leaf maturity on the mangrove leaves (*Sonneratia alba* and *Rhizophora mucronata*) tea powder. *IOP Conf Ser Earth Environ Sci*. 967:012018. doi:10.1088/1755-1315/967/1/012018.
68. Sumartini RPW, Hutabarat RF. 2022. The effect of mangrove types and leaf maturity on tea powder (Sonneratia alba, Rhizophora mucronata). IOP Conf Ser Earth Environ Sci. 967:012018. doi:10.1088/1755-1315/967/1/012018
69. Sundari MS, Sornalakshmi V, Tresina PS, Mohan VR. 2022. Evaluation of phytochemical screening and in vitro anti-inflammatory activity of mangrove associate plant pemphis acidula J.R. Forst and G. Forst (Lythraceae). International journal of health sciences. [accessed 2025 Aug 15]. https://sciencescholar.us/journal/index.php/ijhs/article/view/12879
70. Sun S, Du Y, Li S, Wu C, Zhang M, Li J, Zhang X. 2021. Anti-inflammatory activity of *Chloranthus serratus* in complete Freund's adjuvant-induced arthritic rats. *Experimental and Therapeutic Medicine*. 22:848. https: //doi.org/10.3892/etm.2021.10280
71. Suryaningrum FD. 2021. The Effect Of Mangrove Leaf Extract Dosage Sonneratia Alba On Hela Cell Viability. Journal of Stem Cell Research and Tissue Engineering. 5(1):30. doi:https://doi.org/10.20473/jscrte.v5i1.29383.
72. Surya PRA, Wijaya MD, Udiyani DPC. 2023. Phytochemical screening and antioxidant activity of n-hexane extract of Sonneratia alba mangrove leaves. Biol Med Nat Prod Chem. 12(2):631–635. doi:10.14421/biomedich.2023.122.631-635
73. Vinegar R, Schreiber W, Hugo R. 1969. Biphasic development of carrageenin edema in rats. J Pharmacol Exp Ther. 166(1):96–103.
74. Wijaya MD, Indraningrat AAG, Kirtanayasa IGYA. 2023. Phytochemicals and larvicidal activity of Sonneratia alba root extracts. Biol Med Nat Prod Chem. 12(2):499–505. doi:10.14421/biomedich.2023.122.499-505
75. Winter CA, Risley EA, Nuss GW. 1962. Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. Proc Soc Exp Biol Med. 111(3):544–7.
76. Zhang W, Han J, Li X, An Q, Cao H, Li Y, Guo L, Zhang W, Jin X. 2022. In vivo analgesic, anti-inflammatory and antioxidant potentials of the ethanol extract of Lindera reflexa Hemsl. roots. Sci Rep. 12:16454. Available from: https://www.nature.com/articles/s41598-022-20648-z.
77. Zhang X, Retyunskiy V, Qiao S, et al. 2022. Alloferon-1 ameliorates acute inflammatory responses in λ-carrageenan-induced paw edema in mice. Scientific Reports [Internet]. 12:16689 [cited 2025 Sep 25]. Available from: https://doi.org/10.1038/s41598-022-20648-z

Internet Websites

1. Alfaro RA, MODI PV. 2023 May 22. Diclofenac. PubMed. https://www.ncbi.nlm.nih.gov/books/NBK557879/

2. Benjamin O, Goyal A, Lappin SL. 2023 Jul 3. Disease-modifying antirheumatic drugs (DMARD). In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; [cited 2025 Sep 5]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK507863/
2. Biology Online. 2021 Jul 23. In vivo definition and examples – Biology Online Dictionary. Biology Articles, Tutorials & Dictionary Online. [accessed 2025 Aug 27]. https://www.biologyonline.com/dictionary/in-vivo
3. Biology Online. 2021 July 28). Seed Definition and Examples - Biology Online Dictionary. Biology Articles, Tutorials & Dictionary Online. https://www.biologyonline.com/dictionary/seed
4. Ghlichloo I, Gerriets V. Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) [Updated 2023 May 1]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK547742/
5. Hannoodee S. 2024 Jun 8. Acute inflammatory response. StatPearls [Internet]. https://www.ncbi.nlm.nih.gov/books/NBK556083/
6. Hodgens A, Sharman T. Corticosteroids. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; [cited 2025 Sep 5]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554612/
7. Integrated Taxonomic Information System (ITIS). 2024. Sonneratia alba J. Smith. TSN: 507416 [Internet]. Washington (DC): ITIS. Available from: https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=507416
8. Patel P, Tonog P, Lakhkar AD. Normal Saline. [Updated 2025 Apr 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK545210/
9. Saad J, Mathew D. 2023. Nonsteroidal anti-inflammatory drugs toxicity. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. Available from: https://www.ncbi.nlm.nih.gov/books/NBK526006/
10. Stuart GU Jr. 2024. Pagatpat / Sonneratia alba / Sweet-scented mangrove apple. StuartXchange. https://www.stuartxchange.org/Pagatpat
11. Synapse. 2024 Apr 25. What is LD50? Synapse PATSNAP blog. [accessed 2025 Aug 27]. https://synapse.patsnap.com/blog/what-is-ld50
12. Woodle JM. 2022 Mar 31. 11 ways to reduce inflammation and chronic disease. Assuaged. https://www.assuaged.com/news/11-ways-to-reduce-inflammation-and-chronic-disease

Books

1. Kumar KM, Pola S. 2023. Mangrove species as a potential source of bioactive compounds for diverse therapeutic applications. In: Kim S-K, Shin K-H, Venkatesan JBT-MA, editors. Marine antioxidants. Cambridge (MA): Academic Press. p. 249-263. doi:10.1016/B978-0-323-95086-2.00020-5.
2. Mahmoud AM, Bin-Jumah M, Abukhalil MH. 2021. Antiinflammatory natural products from marine algae. In: Elsevier eBooks. p. 131–159. Available from: https://doi.org/10.1016/b978-0-12-819218-4.00012-2