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# Phytochemical screening and in vitro anti-inflammatory evaluation of *Sechium Edule* fruit extract

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## ABSTRACT
Inflammation is a protective biological process, yet excessive or chronic inflammation can contribute to the development of degenerative and autoimmune diseases. Natural products are increasingly investigated as safer alternatives to synthetic anti-inflammatory drugs. This study aimed to evaluate the phytochemical constituents and in vitro anti-inflammatory activity of Sechium edule (Jacq.) Sw. fruit extract. The fruits were extracted with 70% ethanol by maceration, and qualitative phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, saponins, triterpenoids, and glycosides. Anti-inflammatory activity was determined through inhibition of bovine serum albumin (BSA) and egg albumin denaturation assays at extract concentrations of 100, 200, and 400 µg/mL, using aspirin as a standard. The extract demonstrated concentration-dependent inhibition of BSA denaturation (61.73–69.60%) comparable to aspirin (67.38–71.96%), and moderate inhibition in the egg albumin assay (6.34–27.87% versus 28.32–49.70% for aspirin). These findings suggest that *S. edule* possesses significant anti-inflammatory potential, likely due to the synergistic effects of its polyphenolic and triterpenoid constituents, supporting its traditional medicinal use and indicating its promise as a source of natural anti-inflammatory agents.

***Keywords:*** *Sechium edule, phytochemical screening, anti-inflammatory activity, protein denaturation, albumin assay*

## INTRODUCTION

Inflammation is a fundamental physiological process that protects the body from injury and infection. It serves as a defense mechanism involving cellular and molecular responses that eliminate harmful stimuli and initiate tissue repair (Soliman & Barreda, 2022). However, when this process becomes excessive or chronic, it contributes to the pathogenesis of various degenerative and autoimmune diseases such as rheumatoid arthritis, diabetes mellitus, atherosclerosis, and even cancer. Prolonged inflammation causes tissue damage due to the continuous production of pro-inflammatory mediators, including cytokines, prostaglandins, and reactive oxygen species (Rajendra et al., 2018). Consequently, the development of effective and safe anti-inflammatory agents remains a priority in pharmacological research.

Synthetic anti-inflammatory drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are commonly used to control inflammatory responses. Despite their therapeutic effectiveness, these drugs are often associated with adverse effects such as

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gastric ulceration, renal toxicity, and immunosuppression, particularly when used long-term (Harirforoosh et al., 2013). Therefore, growing attention has been directed toward discovering natural products from medicinal plants as alternative or complementary anti-inflammatory agents. Plant-derived compounds, especially polyphenols, alkaloids, terpenoids, and flavonoids, have demonstrated significant pharmacological properties, including antioxidant, antimicrobial, and anti-inflammatory effects (Elshafie et al., 2023). These natural compounds act through various mechanisms, such as inhibition of cyclooxygenase enzymes, downregulation of inflammatory cytokines, and scavenging of free radicals (Sychrová et al., 2020).

One plant of increasing interest is *Sechium edule* (Jacq.) Sw., commonly known as chayote, belonging to the Cucurbitaceae family. *S. edule* is widely cultivated and consumed as a vegetable in tropical and subtropical regions, including Indonesia. Traditionally, different parts of this plant, such as the fruit, leaves, and roots, have been used in folk medicine to treat hypertension, kidney stones, inflammation, and metabolic disorders (Arista-Ugalde et al., 2022). Phytochemical studies have revealed that *S. edule* contains several bioactive constituents, including flavonoids, saponins, tannins, alkaloids, triterpenoids, and glycosides. These compounds are known to play crucial roles in mediating pharmacological effects through anti-inflammatory, antioxidant, and cytoprotective pathways radicals (Sychrová et al., 2020).

The fruit of *S. edule* has been reported to possess a high content of phenolic compounds and flavonoids, which may contribute to its health-promoting properties. Flavonoids, for instance, are recognized for their ability to inhibit key enzymes in inflammation pathways such as cyclooxygenase (COX) and lipoxygenase (LOX) (Ribeiro et al., 2015), while tannins exert protein-precipitating effects that can stabilize cell membranes and prevent inflammatory damage (Félix-Silva et al., 2014). Saponins and triterpenoids have also been reported to modulate immune responses and suppress the release of inflammatory mediators such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) (Wijesekara et al., 2024). Despite these known pharmacological potentials, scientific evidence supporting the anti-inflammatory activity of *S. edule* fruit extract remains limited, particularly in vitro studies that assess its ability to inhibit protein denaturation.

Protein denaturation is a well-established mechanism involved in inflammation, where proteins lose their native structure and generate antigenic substances that can trigger inflammatory responses (Umapathy et al., 2010). Therefore, preventing protein denaturation is a key therapeutic strategy in the management of inflammation. The albumin denaturation assay has become one of the most widely used in vitro screening methods for evaluating anti-inflammatory activity, as it closely mimics the mechanisms by which drugs stabilize proteins against thermal or chemical stress. In this context, the inhibition of albumin and egg protein denaturation serves as an important preliminary model to evaluate the potential of plant extracts as anti-inflammatory agents.

Considering the rich phytochemical profile and ethnomedicinal importance of *S. edule*, it is essential to investigate its pharmacological potential using a standardized and scientific approach. The exploration of *S. edule* fruit extract not only provides insights into its traditional applications but also supports its potential as a source of natural therapeutic agents for inflammation-related conditions. Furthermore, the global trend toward safer, plant-based

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pharmaceuticals underscores the relevance of studies focusing on edible and widely available plants such as *S. edule*. Its use as both food and medicine presents a valuable opportunity for developing functional foods and nutraceuticals with dual nutritional and pharmacological benefits.

Hence, the present study aims to perform a qualitative phytochemical screening of Sechium edule fruit extract and to evaluate its in vitro anti-inflammatory potential using albumin and egg protein denaturation inhibition assays. Through this investigation, the study seeks to validate the traditional medicinal use of *S. edule* and establish a scientific foundation for future research exploring its bioactive compounds, mechanism of action, and possible clinical applications in inflammatory disorders. The findings are expected to contribute to the growing body of evidence supporting the utilization of natural products in the prevention and treatment of inflammation-associated diseases.

## METHODS

### Ethical Clearance

The study received ethical clearance from the Ethics Committee of the Faculty of Medicine, Universitas Prima Indonesia, Medan, Indonesia (Approval Number:061/KEPK/UNPRI/X/2025).

### Materials

Fresh fruits of Sechium edule were collected from a local market in North Sumatra, Indonesia. Methanol (analytical grade), Bovine Serum Albumin (BSA, Fraction V), phosphate buffer saline (PBS, pH 6.4), and hydrochloric acid (HCl) were obtained from standard laboratory suppliers. Aspirin (acetylsalicylic acid) was used as the reference standard. All other chemicals and reagents used were of analytical grade.

### Preparation of Extract

The fruits of Sechium edule were washed, sliced into small pieces, and shade-dried for several days until a constant weight was achieved. The dried materials were ground into fine powder and extracted with 70% ethanol by maceration for 24 hours with occasional shaking. The filtrate was concentrated using a rotary evaporator under reduced pressure to obtain a thick crude extract, which was stored at 4°C until use (Kemenkes RI, 2017).

### Phytochemical Screening

Qualitative phytochemical tests were conducted following standard protocols to detect the presence of major secondary metabolites. The tests included Mayer's and Dragendorff's reagents for alkaloids, the Shinoda test (Mg–HCl) for flavonoids, ferric chloride (1% $FeCl_3$) test for tannins, the foam test for saponins, the Liebermann–Burchard test for triterpenoids, and the Keller–Kiliani test for glycosides. The intensity or appearance of characteristic color changes or precipitates indicated the presence of respective phytochemicals (Nortjie et al., 2022).

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### Inhibition of Albumin Denaturation Assay

The anti-inflammatory activity of *S. edule* ethanol extract was evaluated based on the inhibition of protein denaturation method as described by Rastogi et al. (2018) with slight modifications. The reaction mixture consisted of equal volumes of test extract at various concentrations (100, 200, and 400 µg/mL) and 1% BSA solution in distilled water. The pH of the mixture was adjusted by adding a few drops of 1N HCl. The samples were incubated at 37°C for 20 minutes, followed by heating at 70°C for 5 minutes. After cooling to room temperature, the absorbance was measured at 660 nm using a UV-Visible spectrophotometer. Aspirin served as the reference drug. The percentage inhibition of protein denaturation was calculated using the following formula:

$$\%\text{Inhibition} = 100 \times (1 - \frac{A_2}{A_1})$$

where $A_1$is the absorbance of the control and $A_2$is the absorbance of the test sample.

### Protein Denaturation (Egg Albumin) Method

The assay was further confirmed following the method of Kiranmayi et al. (2018) with slight modification. The reaction mixture (5 mL total volume) contained 0.2 mL of fresh egg albumin, 2 mL of the test extract at different concentrations (100, 200, and 400 µg/mL), and 2.8 mL of phosphate buffer saline (PBS, pH 6.4). The control received an equal volume of double-distilled water instead of extract. The mixtures were incubated at 37°C for 15 minutes and then heated at 70°C for 5 minutes. After cooling, absorbance was recorded at 660 nm using a spectrophotometer. Aspirin was used as a standard drug. The percentage inhibition was calculated using the formula:

$$\%\text{Inhibition} = \frac{A_{\text{control}} - A_{\text{treated}}}{A_{\text{control}}} \times 100$$

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD).

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## RESULTS

### Phytochemical Constituents

Phytochemical analysis of the ethanol extract of *Sechium edule* fruit revealed the presence of several major classes of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, triterpenoids, and glycosides (Table 1).

Table 1. Phytochemical profile of ethanol extract of *Sechium edule* fruit.

| No | Phytochemical Parameter | Test Method | Observation | Result |
|---|---|---|---|---|
| **1** | Alkaloids | Mayer's and Dragendorff's tests | Formation of yellowish-white precipitate | (+): Present |
| **2** | Flavonoids | Shinoda test (Mg–HCl) | Development of orange-red color | (+): Present |
| **3** | Tannins | Ferric chloride ($FeCl_3$ 1%) test | Formation of dark blue coloration | (+): Present |
| **4** | Saponins | Foam test | Persistent froth observed for more than 10 minutes | (+): Present |
| **5** | Triterpenoids | Liebermann–Burchard test | Greenish-blue color observed | (+): Present |
| **6** | Glycosides | Keller–Kiliani test | Formation of reddish-brown ring | (+): Present |

The diversity of secondary metabolites detected in *S. edule* extract suggests a synergistic contribution to its biological activity. The simultaneous presence of polyphenols (flavonoids and tannins) and triterpenoids may enhance free-radical scavenging capacity and membrane stabilization, both of which are essential for anti-inflammatory activity.

### Inhibition of Albumin Denaturation

The inhibition of protein denaturation assay is a reliable in vitro method to assess the anti-inflammatory potential of compounds. In this study, the ethanol extract of *S. edule* exhibited a concentration-dependent inhibition of albumin denaturation comparable to that of the standard drug, aspirin (Table 2).

Table 2. Inhibition of albumin denaturation by ethanol extract of *Sechium edule* fruit.

| No | Sample | Concentration | Abs ($A_2$) | % Inhibition |
|---|---|---|---|---|
| **A1** | Bovine Serum Albumin (Control) | 1% | 0.546 | — |
| **A2** | Aspirin | 100 µg/mL | 0.178 | 67.38% |
| | | 200 µg/mL | 0.173 | 68.30% |
| | | 400 µg/mL | 0.153 | 71.96% |
| **A2** | *S. edule* Extract | 100 µg/mL | 0.209 | 61.73% |
| | | 200 µg/mL | 0.187 | 65.75% |
| | | 400 µg/mL | 0.166 | 69.60% |

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### Protein Denaturation (Egg Albumin) Assay

The anti-inflammatory activity was further confirmed by evaluating the extract's ability to inhibit egg protein denaturation, another well-established in vitro model. As shown in Table 3, the extract exhibited dose-dependent inhibition, though slightly lower than aspirin. The percentage inhibition for *S. edule* extract ranged from 6.34% at 100 µg/mL to 27.87% at 400 µg/mL, whereas aspirin showed stronger inhibition values between 28.32% and 49.70%.

Table 3. Inhibition of egg protein denaturation by ethanol extract of Sechium edule fruit.

| No | Sample | Concentration | Abs | % Inhibition |
|---|---|---|---|---|
| **Control** | Egg Albumin 4% | — | 0.678 | — |
| **Treated** | Aspirin | 100 µg/mL | 0.486 | 28.32% |
| | | 200 µg/mL | 0.421 | 37.90% |
| | | 400 µg/mL | 0.341 | 49.70% |
| **Treated** | *S. edule* Extract | 100 µg/mL | 0.635 | 6.34% |
| | | 200 µg/mL | 0.563 | 16.95% |
| | | 400 µg/mL | 0.489 | 27.87% |

## DISCUSSION

The phytochemical analysis indicated the bioactive constituents are well-known for their pharmacological significance, particularly in exerting antioxidant, antimicrobial, and anti-inflammatory effects. The positive results obtained from Mayer's and Dragendorff's tests confirmed the presence of alkaloids, which play essential roles in modulating pain and inflammation through inhibition of prostaglandin synthesis and suppression of inflammatory cytokines (Shoaib et al., 2016).

Flavonoids, detected through the Shinoda test, developed an orange-red color indicating their abundance in the extract. Flavonoids have been extensively documented as potent anti-inflammatory agents capable of reducing oxidative stress and suppressing enzymes such as cyclooxygenase (COX) and lipoxygenase (LOX), which are responsible for prostaglandin and leukotriene synthesis (Tunon, et al., 2009). The presence of tannins, confirmed by the formation of a dark blue coloration with ferric chloride, suggests additional anti-inflammatory potential, since tannins possess protein-precipitating properties that can stabilize biological membranes and prevent inflammatory damage.

Saponins were identified by the persistent froth observed for more than ten minutes in the foam test. These compounds are known to inhibit histamine release and reduce tissue edema, thereby contributing to the suppression of inflammatory pathways (Choi et al., 2005). The Liebermann–Burchard test revealed a greenish-blue coloration, indicating triterpenoids,

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which are associated with inhibition of nitric oxide production and modulation of nuclear factor-κB (NF-κB) activity (Chen et al., 2019)

At concentrations of 100, 200, and 400 µg/mL, the extract produced inhibition values of 61.73%, 65.75%, and 69.60%, respectively, whereas aspirin exhibited slightly higher inhibition ranging from 67.38% to 71.96%. The progressive increase in percentage inhibition with higher concentrations indicates that the extract effectively stabilizes protein structures against heat-induced denaturation, which mimics the mechanism of anti-inflammatory drugs that protect proteins from inflammatory stress.

The high inhibitory activity observed may be attributed to the synergistic effects of flavonoids and tannins present in the extract. These compounds are known to bind to proteins through hydrogen bonding, preventing unfolding and aggregation caused by heat. Similar findings were reported by Rastogi et al. (2018), who demonstrated that plant polyphenols can act as efficient stabilizers of serum albumin. The ability of *S. edule* extract to inhibit protein denaturation nearly equivalent to aspirin suggests that it contains bioactive components with potential anti-inflammatory mechanisms. Moreover in eeg albumin assay, although the inhibition values were lower than the standard drug, the increasing trend with concentration suggests that the extract possesses moderate protein-stabilizing potential. The reduced activity in this assay may be due to differences in protein composition between egg albumin and bovine serum albumin, as egg proteins are more complex and less susceptible to stabilization by polyphenolic compounds. Nevertheless, the observed activity aligns with previous reports that extracts containing flavonoids, saponins, and triterpenoids can reduce inflammation by protecting proteins from denaturation and aggregation (Enechi et al., 2020). These findings suggest that *S. edule* fruit extract exerts its anti-inflammatory effect primarily through membrane stabilization and prevention of thermal-induced protein alteration, similar to the mode of action of NSAIDs.

Overall, the results indicate that Sechium edule fruit extract exhibits significant anti-inflammatory potential through inhibition of protein denaturation. The presence of diverse phytochemicals supports its use in traditional medicine for inflammation management and provides a scientific foundation for further investigation into its bioactive components, molecular mechanisms, and in vivo efficacy.

## CONCLUSION

The ethanol extract of *Sechium edule* fruit demonstrated significant in vitro anti-inflammatory activity by inhibiting protein denaturation in both albumin and egg protein models. Phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, saponins, triterpenoids, and glycosides, which may act synergistically to stabilize proteins and reduce inflammatory responses. The extract's activity, comparable to aspirin at higher concentrations, supports its traditional use in treating inflammation. Further studies involving isolation of active compounds and in vivo evaluations are recommended to elucidate its precise mechanisms and therapeutic potential.

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