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BSA Protein Denaturation Inhibition by *Averrhoa bilimbi* L. Fruit Ethanol Extract: In Vitro Study

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ABSTRACT

Inflammation is a protective physiological response; however, excessive or persistent inflammation may contribute to tissue dysfunction and disease. Plant-derived secondary metabolites have been widely explored as potential anti-inflammatory agents with fewer long-term adverse effects than conventional drugs. This study investigated the phytochemical profile and in vitro anti-inflammatory activity of the 96% ethanol extract of *Averrhoa bilimbi* L. fruit using the bovine serum albumin (BSA) protein denaturation inhibition assay. The extract was obtained by maceration, followed by qualitative tube-based phytochemical screening. Anti-inflammatory activity was evaluated at final assay concentrations of 20, 40, 60, 80, and 100 ppm, with sodium diclofenac as a positive control (10–40 ppm). The mixture containing BSA was incubated at 25 °C for 30 min, heated at ~72 °C for 5 min, and absorbance was measured at 660 nm. Phytochemical screening indicated the presence of flavonoids, alkaloids, and tannins. The extract inhibited protein denaturation in a concentration-dependent manner with inhibition percentages of 20.67%, 45.33%, 50.13%, 55.14%, and 60.22% (20–100 ppm), yielding an IC_{50} of 60.94 µg/mL (≈ppm). Sodium diclofenac showed stronger inhibition with an IC_{50} of 8.20 µg/mL. These findings support the phytochemical potential of *A. bilimbi* fruit extract as a preliminary in vitro anti-inflammatory candidate, warranting further fractionation and mechanistic studies to identify active constituents and confirm activity beyond this screening model.

Keywords:
Averrhoa bilimbi L.; Phytochemical screening; Anti-inflammatory; BSA protein denaturation assay; Ethanol extract; IC_{50}

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1. Introduction

The use of natural resources as traditional medicine remains deeply embedded in Indonesian society and continues to be transmitted across generations as a pragmatic approach to health maintenance and management of common illnesses [1]. In parallel with this cultural continuity, systematic documentation and scientific substantiation of medicinal plants are increasingly important to ensure that traditional practices remain rational, safe, and evidence-informed. At the national level, the strengthening of herbal utilisation is also reflected in policy instruments such as the Indonesian Traditional

Herbal Medicine Formulary, which indicates a strategic commitment to integrating evidence-based herbal resources within health services [2].

Inflammation is a protective biological response to tissue injury and cellular disturbance, yet excessive or persistent inflammation may contribute to pathological conditions that compromise tissue function and quality of life. Within this context, plant secondary metabolites, particularly polyphenolic compounds such as flavonoids, have attracted major attention due to their broad bioactivities, including inflammation-related modulation and antioxidant-associated mechanisms [3]. Therefore, the exploration of phytochemical-rich botanicals as preliminary anti-inflammatory candidates remains a relevant research direction, especially when positioned as an initial screening step prior to more definitive pharmacological evaluation.

Averrhoa bilimbi L. (belimbing wuluh) is widely recognised and utilised in daily life, primarily as a culinary ingredient due to its distinctive sour taste, and it is also described in local health-education sources as a plant with traditional therapeutic relevance plants [4]. Empirical use alone is insufficient to infer efficacy; nevertheless, it provides a rational basis for scientific investigation of bioactive potential. Previous work has reported that ethanol extracts of *A. bilimbi* exhibit measurable anti-inflammatory activity using a protein denaturation inhibition approach, supporting the plausibility that this species contains constituents capable of modulating inflammation-related biochemical processes [5].

Protein denaturation involves conformational alteration of a protein's higher-order structure that leads to functional impairment without cleavage of peptide bonds. Because denaturation can be induced under stress conditions and is associated with inflammatory progression, inhibition of protein denaturation has been widely applied as a practical *in vitro* proxy for screening anti-inflammatory potential of plant extracts, including through the bovine serum albumin (BSA) denaturation model [6]. In this assay, activity is typically expressed as percent inhibition and summarised as an IC_{50} value to facilitate relative comparisons across extracts and reference drugs [7]. Evidence from multiple botanical studies employing the same assay framework further supports its methodological relevance as an initial screening platform for anti-inflammatory potential [8],[9].

Therefore, this study was conducted to (i) characterise the phytochemical profile of the 96% ethanol extract of *A. bilimbi* fruit using qualitative phytochemical screening and (ii) evaluate its *in vitro* anti-inflammatory activity using the BSA protein denaturation inhibition assay, with sodium diclofenac serving as a comparative positive control within a standardised screening system.

2. Methods

Study design

This study employed a laboratory experimental design consisting of (i) qualitative phytochemical screening of the 96% ethanol extract of *Averrhoa bilimbi* L. fruit and (ii) evaluation of *in vitro* anti-inflammatory activity using the bovine serum albumin (BSA) protein denaturation inhibition assay. The overall workflow and assay rationale were adapted from prior botanical anti-inflammatory screening studies using protein denaturation as an early proxy model, with sodium diclofenac as a comparative reference drug [5],[9],[10].

Materials and instruments

Fresh *A. bilimbi* fruits were used as the raw material. Analytical and laboratory-grade reagents included 96% ethanol, BSA, Tris buffer saline (TBS) components (Tris

base, NaCl), glacial acetic acid (for pH adjustment), and distilled water. Phytochemical screening reagents included Dragendorff reagent (alkaloids), Mg powder and concentrated HCl (flavonoids), $FeCl_3$ (tannins), and Liebermann–Burchard reagent (steroids/terpenoids), consistent with phytochemical screening practices commonly applied in related botanical anti-inflammatory studies [11],[12]. A UV–Vis spectrophotometer and standard glassware were used for absorbance measurement and solution preparation, following routine spectrophotometric analytical practices [14].

Sample preparation and extraction

Fruits were washed, sliced, dried under controlled conditions, and milled to obtain simplicia powder. A total of 300 g simplicia powder was macerated using 96% ethanol (1,000 mL) with complete immersion in a closed container for 72 h, with intermittent agitation (15 min every 24 h). The filtrate was collected and concentrated under reduced pressure using a rotary evaporator at 40 °C to obtain a viscous crude extract. Extraction yield was calculated as percentage yield using the standard formula:

$$\% \text{ Yield} = \frac{\text{Weight of concentrated extract}}{\text{Weight of dried simplicia}} \times 100$$

This yield calculation approach is routinely used in botanical extract studies and is reported in comparable methodological workflows [12],[13].

Phytochemical screening

Qualitative phytochemical screening was performed using tube tests to identify major secondary metabolite classes. Flavonoids were assessed by adding Mg powder and concentrated HCl to the extract solution, with a red/orange colour change indicating positivity. Alkaloids were tested by acidifying the extract with HCl, heating, filtering, and adding Dragendorff reagent to the filtrate; formation of turbidity/precipitate indicated alkaloid presence. Tannins were evaluated using 1% $FeCl_3$, where a dark green/blackish coloration indicated positivity. Saponins were assessed via foam formation after vigorous shaking with hot water (persistent foam after addition of dilute acid was considered positive). Steroids/terpenoids were assessed using Liebermann–Burchard reagent with characteristic ring/colour development. These qualitative screens align with phytochemical profiling approaches applied in anti-inflammatory screening studies of botanical extracts [11],[12].

Preparation of buffers and protein solution

Tris buffer saline (TBS) was prepared by dissolving 8.7 g NaCl and 1.21 g Tris base in distilled water, adjusting pH to 6.2–6.5 using glacial acetic acid, and making up to 1,000 mL with distilled water, consistent with an acidic assay environment used in protein denaturation-based anti-inflammatory screening [8]. A 0.2% BSA solution was prepared by dissolving 0.2 g BSA in TBS and making up to 100 mL [5].

Preparation of test, negative control, and positive control solutions

The negative control consisted of 50 µL ethanol added to 0.2% BSA and made up to 5 mL with TBS/BSA solution (BSA + solvent system) [10]. For the positive control, sodium diclofenac stock solution was prepared by dissolving 100 mg sodium diclofenac in methanol and making up to 25 mL to obtain 4,000 ppm. Working solutions were prepared at 1,000; 2,000; 3,000; and 4,000 ppm to achieve final assay concentrations of 10; 20; 30; and 40 ppm after reaction dilution [9],[10].

For the test extract, 500 mg crude extract was dissolved in ethanol and made up to 25 mL to obtain a 20,000 ppm stock solution. Working solutions were prepared at

2,000; 4,000; 6,000; 8,000; and 10,000 ppm to achieve final assay concentrations of 20; 40; 60; 80; and 100 ppm, respectively, after reaction dilution.

BSA protein denaturation inhibition assay

For each treatment, 50 µL of the working solution (extract or diclofenac) was mixed with 0.2% BSA and adjusted to a final volume of 5 mL, yielding the final concentrations described above. The mixtures were incubated at 25 °C for 30 min, then heated at ~72 °C for 5 min in a water bath to induce denaturation, cooled at room temperature for 25 min, and gently mixed prior to measurement. Absorbance was measured at 660 nm using a UV–Vis spectrophotometer. This assay framework follows the protein denaturation inhibition model widely used for *in vitro* anti-inflammatory screening of botanical extracts [5], [7]–[12].

Calculation of percent inhibition and IC_{50}

Protein denaturation inhibition was calculated using:

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

where $A_{\text{control}}$is the absorbance of the negative control (BSA + solvent) and $A_{\text{sample}}$is the absorbance of the treatment mixture. IC_{50} (the concentration producing 50% inhibition) was estimated by linear regression of percent inhibition versus concentration and interpolating the concentration at 50% inhibition, consistent with calculation procedures used in related denaturation-based studies [7], [13].

Statistical analysis

Phytochemical screening outcomes were reported descriptively. Quantitative inhibition data were summarised as mean ± SD from replicate measurements. For comparisons among multiple concentrations, one-way ANOVA with an appropriate post-hoc test ($\alpha = 0.05$) was applied to evaluate differences across groups, as commonly adopted in comparable *in vitro* anti-inflammatory screening studies [7], [9], [11].

3. Results and Discussion

Characteristics of Raw Material, Extraction Output, and Yield

The raw material comprised *Averrhoa bilimbi* L. fruits collected from Modelomo Village, Tilamuta District, Boalemo Regency, Gorontalo Province, Indonesia. After collection, the fruits were washed to remove adhering contaminants, sliced to facilitate moisture removal, and dried to obtain simplicia prior to milling into a homogeneous powder for extraction. The extraction was performed by maceration using 96% ethanol, yielding a concentrated crude extract suitable for subsequent phytochemical screening and the BSA protein denaturation assay, consistent with botanical anti-inflammatory screening workflows that commonly employ ethanol maceration as an initial extraction approach for complex plant matrices [5].

Table 1. Extraction output and yield of *Averrhoa bilimbi* L. fruit ethanol extract

Solvent	Solvent Volume (mL)	Dried Simplicia (g)	Concentrated Extract (g)	Yield (%)
Ethanol (96%)	1000	300	78	26

A total of 300 g dried fruit simplicia extracted with 1,000 mL ethanol produced 78 g of concentrated extract, corresponding to a 26% yield (**Table 1**). Percentage yield is a practical extraction-performance indicator, reflecting the mass fraction of concentrated

extract recovered from the starting dried simplicia under a defined solvent system and process conditions, and it is routinely reported in comparable botanical extraction studies to contextualise downstream bioassay outcomes [12], [13]. In the present study, the relatively substantial yield suggests that 96% ethanol effectively solubilised a broad fraction of extractable constituents from the dried fruit matrix, thereby providing adequate extract mass for reproducible phytochemical screening and concentration–response testing in the anti-inflammatory assay.

Phytochemical Screening Results of A. bilimbi Fruit Ethanol Extract

Qualitative phytochemical screening demonstrated that the 96% ethanol extract of *Averrhoa bilimbi* L. fruit contained flavonoids, alkaloids, and tannins, while saponins, terpenoids, and steroids were not detected under the applied tube-test conditions (Table 2). The detection of flavonoids and tannins is pharmacologically relevant because polyphenolic classes are frequently discussed as bioactive secondary metabolites with inflammation-related modulatory potential in medicinal-plant research [3]. The presumptive presence of alkaloids may also support bioactivity plausibility, although qualitative assays do not resolve alkaloid identity or abundance and therefore cannot be used to infer potency without further analytical confirmation [12],[15].

Table 2. Phytochemical screening results of *Averrhoa bilimbi* L. fruit ethanol extract

Compound Class	Reagent	Test Result	Remarks
Flavonoids	Magnesium (Mg) and HCl	Color change observed	Positive (+)
Alkaloids	Dragendorff reagent	Orange precipitate	Positive (+)
Saponins	Warm water and HCl	No foam formed	Negative (–)
Tannins	Ferric chloride ($FeCl_3$)	Blackish green	Positive (+)
Terpenoids	Liebermann–Burchard reagent	No color change	Negative (–)
Steroids	Liebermann–Burchard reagent	No color change	

These results should be interpreted as presence/absence indications based on colour or precipitate formation rather than definitive compositional profiling. Accordingly, the chemical–bioactivity linkage in the present study remains hypothesis-generating, and future work would be strengthened by quantitative standardisation (e.g., total phenolics/total flavonoids) and chromatographic fingerprinting to improve interpretability and reproducibility [7],[11].

BSA Protein Denaturation Inhibition Assay

The *in vitro* anti-inflammatory activity of *Averrhoa bilimbi* L. fruit ethanol extract was evaluated using the BSA protein denaturation inhibition model, in which lower absorbance relative to the negative control (BSA + solvent) indicates greater inhibition of thermally induced protein denaturation. This assay has been widely applied as an initial screening proxy to appraise anti-inflammatory potential of botanical extracts and to enable comparison against a reference NSAID such as sodium diclofenac [5]–[8], [10]. In the present dataset, the extract produced a clear concentration-dependent inhibitory pattern, with inhibition increasing from 20.6686% at 20 ppm to 60.2163% at 100 ppm (**Table 3**). Concomitantly, absorbance decreased from 1.3580 (20 ppm) to 0.6743 (100 ppm), indicating progressive suppression of denaturation-associated turbidity as the extract concentration increased.

Table 3. Anti-inflammatory activity of *Averrhoa bilimbi* L. fruit ethanol extract and sodium diclofenac using the BSA protein denaturation assay

Sample	Concentration (ppm)	Absorbance	% Inhibition	IC_{50} (µg/mL)
Sodium diclofenac	10	0.7320	56.8142	8.2040
Sodium diclofenac	20	0.6657	60.7276	8.2040
Sodium diclofenac	30	0.4687	72.3500	8.2040
Sodium diclofenac	40	0.2837	83.2645	8.2040
***A. bilimbi* fruit ethanol extract**	20	1.3580	20.6686	60.9357
***A. bilimbi* fruit ethanol extract**	40	0.9267	45.3294	60.9357
***A. bilimbi* fruit ethanol extract**	60	0.8453	50.1278	60.9357
***A. bilimbi* fruit ethanol extract**	80	0.7603	55.1426	60.9357
***A. bilimbi* fruit ethanol extract**	100	0.6743	60.2163	60.9357

In contrast, sodium diclofenac exhibited stronger inhibition across the tested range, achieving 56.8142% inhibition already at 10 ppm, and rising to 83.2645% at 40 ppm (**Table 3**). The substantially lower absorbance values for diclofenac (0.7320 at 10 ppm to 0.2837 at 40 ppm) are consistent with a potent stabilizing effect on protein conformation under heat-induced denaturation conditions, which is congruent with its established anti-inflammatory pharmacology and its frequent use as a positive control in denaturation-based screening studies [6],[7],[10]. Collectively, these results indicate that the *A. bilimbi* fruit ethanol extract possesses measurable anti-denaturation activity, yet its inhibitory performance remains quantitatively weaker than diclofenac within the tested concentration windows, supporting its interpretation as a preliminary, moderate-intensity candidate within this screening framework [7], [8].

Dose–Response Relationship and IC_{50} Estimation

A concentration–response pattern was observed for both the *Averrhoa bilimbi* L. fruit ethanol extract and the positive control, sodium diclofenac, indicating that increasing concentrations progressively enhanced inhibition of heat-induced BSA denaturation (**Table 3**). For the extract, inhibition increased from 20.6686% at 20 ppm to 60.2163% at 100 ppm, demonstrating a monotonic trend consistent with dose-dependent anti-denaturation behaviour reported in botanical screening studies employing this model [7],[8]. Sodium diclofenac exhibited higher inhibition within a lower concentration window (10–40 ppm), reflecting its expected stronger inhibitory capacity in this assay system [6], [10].

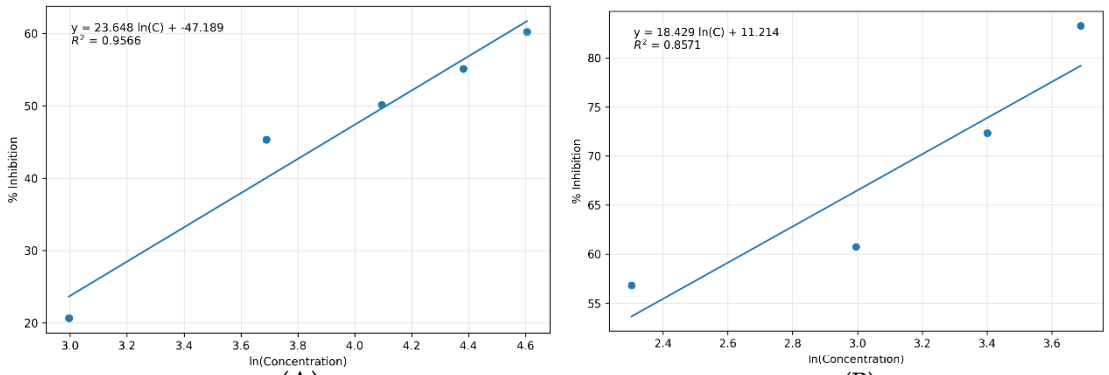

Figure 1. Semi-log linear regression plots of percent inhibition versus ln(concentration) in the BSA protein denaturation assay: (A) *Averrhoa bilimbi* L. fruit ethanol extract (20–100 ppm) and (B) sodium diclofenac (10–40 ppm).

To estimate IC_{50}, a semi-log linear regression approach was applied by plotting inhibition percentage against ln(concentration), as presented in **Figure 1** (extract and diclofenac). The regression equations were subsequently used to interpolate the concentration corresponding to 50% inhibition, which operationally defines IC_{50} within the tested range. For the extract, the regression relationship showed a strong fit to the semi-log model (Figure 1), supporting the appropriateness of using the fitted line for interpolation under the present concentration interval. For diclofenac, the semi-log model also produced an interpretable dose–response relation (Figure 2), although with a comparatively lower fit, which may reflect the narrower concentration range and steeper pharmacodynamic response typical for potent NSAIDs in protein-stabilisation screening models [7],[10].

Based on the regression-derived interpolation, the IC_{50} of the *A. bilimbi* fruit ethanol extract was 60.9357 µg/mL (ppm under dilute aqueous assay conditions), whereas sodium diclofenac yielded a substantially lower IC_{50} of 8.2040 µg/mL (**Table 3**). The marked difference in IC_{50} values indicates that diclofenac achieved the 50% inhibitory threshold at considerably lower concentrations than the crude fruit extract, which is consistent with its established anti-inflammatory potency and its role as a reference standard in denaturation-based screening studies [6], [7], [10].

Nevertheless, the extract's IC_{50} near ~61 µg/mL remains a meaningful initial indicator of measurable anti-denaturation activity, particularly because crude extracts comprise complex mixtures in which active constituents may be diluted by non-active matrix components. This interpretation is aligned with evidence from related denaturation-based studies, where crude botanical extracts frequently exhibit moderate IC_{50} values and may demonstrate enhanced activity following fractionation and enrichment of dominant bioactive constituents [8], [11], [12]. Importantly, the regression fit (e.g., R^2) should be interpreted as an indicator of model adequacy for the observed data rather than as a direct measure of biological effectiveness; therefore, the most defensible conclusion from Figure 1–2 is that both the extract and diclofenac exhibit concentration-dependent inhibition within the tested conditions, with IC_{50} values that enable relative comparison of inhibitory strength in this screening system [7].

Statistical Interpretation Across Concentrations

Statistical inference was applied to strengthen the interpretation that the observed inhibitory responses were not attributable to random analytical fluctuation

alone. In the current dataset, the summary statistics indicate that the absorbance values obtained from the sodium diclofenac groups were consistently lower than those from the *Averrhoa bilimbi* L. fruit ethanol extract groups (**Table 4**), reflecting stronger suppression of heat-induced BSA denaturation by the reference NSAID within the tested concentration range. The reported significance level should be expressed as **p < 0.001** rather than "p = 0.000," because p-values represent probabilities and are conventionally reported using inequality notation when they fall below a rounding threshold.

Table 4. Summary statistics of absorbance values in the BSA protein denaturation assay for *Averrhoa bilimbi* L. fruit ethanol extract and sodium diclofenac

Group	N	Mean ± SD (Absorbance)	p-value
Averrhoa bilimbi L. fruit ethanol extract	18	1.043 ± 0.374	p < 0.001
Sodium diclofenac	12	0.537 ± 0.184	p < 0.001

Nevertheless, for methodological consistency, it is critical to align the statistical variable with the biological endpoint of the assay. Because anti-inflammatory activity in the BSA model is interpreted through percent inhibition, the most defensible approach is to perform one-way ANOVA using % inhibition as the dependent variable for each set of concentrations (extract: 20–100 ppm; diclofenac: 10–40 ppm), followed by an appropriate post-hoc test to determine which concentration pairs differ significantly. This approach is preferable to analysing raw absorbance alone, because % inhibition normalises each measurement to the negative control and directly represents the functional outcome of protein stabilisation under thermal stress. Moreover, post-hoc testing is essential when multiple concentrations are compared to control the family-wise error rate and avoid inflated false-positive conclusions. Such an analytical strategy is consistent with statistical practice in comparable denaturation-based *in vitro* anti-inflammatory screening studies [7],[9],[11].

Accordingly, the statistical interpretation in this study should be framed as follows: concentration increases produced progressively higher inhibition for the extract and diclofenac, and formal group comparison using ANOVA on % inhibition is expected to support significant between-concentration differences under the present design, thereby reinforcing the dose–response interpretation. Importantly, statistical significance should be interpreted alongside biological relevance, where the IC_{50} values provide the most informative comparative summary: the crude fruit extract reached the 50% inhibition threshold at approximately 60–61 µg/mL, whereas sodium diclofenac reached the same threshold at substantially lower concentrations, consistent with its higher inhibitory potency in this screening framework [7],[10].

Comparative Previous Studies and Mechanistic Plausibility

The present findings indicate that the 96% ethanol extract of *Averrhoa bilimbi* L. fruit exhibits measurable anti-denaturation activity in the BSA protein denaturation assay, with inhibition increasing in a concentration-dependent manner and an IC_{50} of 60.9357 µg/mL (**Table 3**; **Figure 1**). This outcome is broadly consistent with the literature that employs protein denaturation inhibition as an initial *in vitro* proxy for anti-inflammatory potential of botanical extracts, where crude extracts commonly demonstrate moderate inhibitory strength that can be benchmarked against an NSAID reference such as sodium diclofenac [7],[9],[11],[12]. Within this methodological context, the markedly lower IC_{50} of diclofenac (8.2040 µg/mL; Figure 2) is expected and

reinforces that the fruit extract should be interpreted as a preliminary candidate rather than an equivalent substitute within this screening system [10].

A particularly relevant comparison is provided by the prior study on *A. bilimbi* using protein denaturation inhibition, which reported anti-inflammatory activity for ethanol extracts of the plant (notably leaves) and thereby supports the plausibility that this species contains constituents capable of modulating denaturation-associated inflammatory proxies [5]. Differences between the present fruit-based extract and leaf-based studies are scientifically plausible because metabolite distribution is organ-dependent and can vary substantially between fruits and leaves, which may contribute to differences in inhibition magnitude and IC_{50} estimates across studies even when the same assay principle is applied [5], [7]. Moreover, the present phytochemical screening identified flavonoids and tannins, both of which are widely recognised as polyphenolic classes associated with anti-inflammatory and antioxidant-related bioactivities in medicinal plants [3]. Although the qualitative nature of phytochemical screening precludes quantitative attribution, these classes provide a coherent chemical rationale for the observed activity, as polyphenolic compounds can interact with proteins and potentially stabilise conformation under denaturing conditions, thereby reducing turbidity formation in the BSA assay [7],[9].

Beyond *A. bilimbi*, multiple botanical studies using the same BSA denaturation framework have reported concentration-dependent inhibition and the derivation of IC_{50} values as comparative potency metrics. For instance, anti-denaturation activity has been documented for ethanol extracts and fractions from diverse plant sources, supporting the methodological relevance of the assay as an early screening platform and indicating that crude extracts may show improved activity after fractionation or formulation-based enrichment [9], [11], [12]. Collectively, these comparisons position the present findings within an established screening literature: the *A. bilimbi* fruit extract demonstrates reproducible dose-dependent inhibition, yet its inhibitory strength remains lower than the reference NSAID, thereby justifying further work focused on standardisation, fractionation, and mechanistic confirmation beyond this proxy model [7],[11],[12].

Study Limitations and Implications for Future Work

his study has several limitations that should be considered when interpreting the findings. The BSA protein denaturation assay represents an *in vitro* screening proxy that reflects the ability of a sample to suppress thermally induced protein conformational changes, but it does not directly confirm anti-inflammatory efficacy in complex biological systems. In addition, the phytochemical evaluation was qualitative and restricted to presence/absence identification of major metabolite classes, so the specific active constituents and their relative contributions to the observed inhibition cannot be determined from the current dataset. The use of a crude extract also implies that the measured activity may be influenced by matrix components and assay conditions, while the IC_{50} estimation is inherently dependent on the tested concentration range and regression assumptions. Accordingly, future work should prioritise extract standardisation (e.g., quantitative phenolic/flavonoid indices), chromatographic fingerprinting, and bioassay-guided fractionation to identify enriched active fractions, followed by confirmatory mechanistic assays and, where feasible, validation in cellular or in vivo inflammation models to strengthen translational relevance.

4. Conclusions

he 96% ethanol extract of *Averrhoa bilimbi* L. fruit demonstrated the presence of flavonoids, alkaloids, and tannins based on qualitative phytochemical screening. In the BSA protein denaturation inhibition assay, the extract exhibited concentration-

dependent anti-denaturation activity across 20–100 ppm, reaching 60.2163% inhibition at 100 ppm and yielding an IC_{50} of 60.9357 µg/mL, whereas sodium diclofenac showed stronger inhibition with an IC_{50} of 8.2040 µg/mL. Overall, these results indicate that *A. bilimbi* fruit ethanol extract has measurable *in vitro* anti-inflammatory potential within this screening model, although its inhibitory strength remains lower than the reference NSAID, thereby supporting its role as a preliminary candidate for further standardisation and bioactivity-focused investigation.

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Conflicts of Interest:
The author declares that there are no conflicts of interest in this research.

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