Journal Syifa Sciences and Clinical Research (JSSCR)
Volume 8 Number 1, 2026
Journal Homepage: http://ejurnal.ung.ac.id/index.php/jsscr, E-ISSN: 2656-9612 P-ISSN:2656-8187
DOI: https://doi.org/10.37311/jsscr.v8i1.36345

Phytochemical Screening and In Vitro Anti-Inflammatory Activity of *Andrographis paniculata* Leaves via BSA Denaturation

Hamsidar Hasan[1*], Nurain Thomas[2], A. Mu'thi Andy Suryadi[3], Endah Nurrohwinta Djuwarno[4], Fitri Nento[5]

[1,2,3,4,5] *Department of Pharmacy, Faculty of Sports and Health, Gorontalo State University, Jenderal Sudirman Street 06 Gorontalo City, 96128, Indonesia*

**Corresponding author. Email: hamsidar.hasan@ung.ac.id*

ABSTRACT

Inflammation is a protective biological response to tissue injury; however, excessive or persistent inflammation may contribute to pathological conditions. *Andrographis paniculata* leaves (king of bitters) are widely used in traditional medicine, yet systematic screening of their anti-inflammatory potential remains necessary. This study aimed to characterise the phytochemical profile and evaluate the *in vitro* anti-inflammatory activity of a 96% ethanolic leaf extract using the bovine serum albumin (BSA) protein denaturation inhibition assay. Dried leaf powder (300 g) was macerated with 96% ethanol to obtain a concentrated extract with a yield of 14% (w/w). Qualitative screening indicated the presence of alkaloids, flavonoids, tannins, saponins, and terpenoids, while steroids were not detected. Anti-inflammatory activity was assessed at 5–25 ppm, with sodium diclofenac as a positive control. The extract exhibited a concentration-dependent increase in inhibition, from 21.30% (5 ppm) to 72.82% (25 ppm), and produced an IC_{50} of 14.1467 ppm. Overall, the ethanolic leaf extract of *A. paniculata* demonstrated measurable anti-denaturation activity in this screening model, supporting its potential as a candidate for further standardisation and confirmatory anti-inflammatory testing.

Keywords: *Andrographis paniculata*; Anti-inflammatory; Bovine serum albumin; Protein denaturation; Phytochemical screening

Received:	*Accepted:*	*Online:*
2025-11-22	*2026-01-26*	*2026-01-31*

1. Introduction

Inflammation is a fundamental protective response to tissue injury and infection; however, uncontrolled or persistent inflammatory processes may contribute to chronic pathological conditions. In many settings, the community continues to rely on medicinal plants as complementary approaches to manage inflammatory symptoms, driven by cultural acceptance, accessibility, and perceived safety. One such medicinal plant is king of bitters (*Andrographis paniculata*), which has long been utilised in Indonesia and other regions as traditional herbal medicine. Empirically, *A. paniculata* leaves are used for wound-related conditions and are commonly associated with antimicrobial and inflammation-related benefits, consistent with reports describing broad pharmacological potential attributable to andrographolide, flavonoids, and other secondary metabolites [1,2].

Beyond ethnomedicinal utilisation, experimental and clinical evidence has positioned *A. paniculata* as a bioactive botanical with diverse pharmacological activities, including immunomodulatory, antioxidant, anti-inflammatory, antidiabetic,

hepatoprotective, antihypertensive, and antimicrobial effects [3,4]. Among its key constituents, andrographolide has been recognised as a major diterpenoid lactone with plausible anti-inflammatory mechanisms. Mechanistically, andrographolide and related constituents have been linked to modulation of inflammatory signalling, including inhibition of iNOS and suppression of NF-κB–related pathways, thereby reducing downstream production of pro-inflammatory mediators such as NO and cytokines (e.g., TNF-α and IL-1) that contribute to inflammatory cell recruitment and tissue injury [5].

From a methodological perspective, *in vitro* screening approaches remain important for early-phase evaluation of anti-inflammatory potential because they are time-efficient, require small sample volumes, and avoid the ethical and logistical constraints of animal experiments. One widely applied proxy approach is the protein denaturation inhibition assay, particularly using bovine serum albumin (BSA), where inhibition of heat-induced denaturation is interpreted as an indicator of protein-stabilising capacity relevant to inflammatory processes. Recent methodological work has summarised assay conditions and optimisation considerations for BSA denaturation–based anti-inflammatory screening, supporting its utility as an initial bioactivity filter prior to more specific mechanistic assays [6]. Complementary denaturation models, including egg albumin denaturation and BSA denaturation across diverse botanical extracts, further reinforce that denaturation-inhibition assays can provide reproducible concentration–response data for preliminary comparison between extracts and reference drugs [7,8].

Therefore, given the established bioactive profile of *A. paniculata* and the practicality of BSA denaturation as an early screening platform, this study was designed to (i) characterise the phytochemical profile of the 96% ethanolic leaf extract of *A. paniculata* and (ii) evaluate its *in vitro* anti-inflammatory activity by assessing inhibition of BSA protein denaturation using UV–Visible spectrophotometry, with sodium diclofenac as a positive control comparator. This approach is expected to provide a defensible preliminary estimate of inhibitory strength (including IC_{50}) and to support subsequent standardisation and confirmatory testing in more biologically informative inflammation models.

2. Methods

Study design

This study employed a laboratory experimental design to evaluate the *in vitro* anti-inflammatory activity of *Andrographis paniculata* leaves using a protein denaturation inhibition assay (BSA model).

Materials and instruments

Materials included distilled water, aluminium foil, 70% ethanol, *A. paniculata* leaf extract, filter paper, 96% ethanol, ethyl acetate, $FeCl_3$, concentrated HCl, H_2SO_4, magnesium metal, 0.9% NaCl, n-hexane, Dragendorff reagent, Mayer reagent, Liebermann–Burchard reagent, silica gel, bovine serum albumin (BSA), sodium diclofenac, and Tris buffer saline. Instruments comprised standard glassware, maceration vessel, blender, rotary evaporator, analytical balance, micropipettes, volumetric flasks, test tubes, and a UV–Vis spectrophotometer.

Sample preparation and extraction

Powdered leaves (300 g) were extracted with 96% ethanol using total maceration. The sample was immersed in 2 L ethanol for 3 × 24 h and subsequently filtered. The filtrate was concentrated using an evaporator to obtain a viscous ethanolic extract,

following the referenced maceration procedure [9]. Extraction yield was calculated using:

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

Phytochemical screening

Alkaloid test. A total of 0.1 g extract was mixed with 5 mL chloroform and 3 drops ammonia. The chloroform layer was separated and acidified with 2 drops H_2SO_4. The acid fraction was divided into three tubes and treated separately with Dragendorff, Mayer, and Wagner reagents. Alkaloids were indicated by precipitate formation (white with Mayer, red with Dragendorff, and brown with Wagner) [10].

Tannin test. A total of 0.1 g extract was mixed with distilled water until homogeneous. Five drops of 1% $FeCl_3$ were added, and dark green coloration indicated tannins [11].

Flavonoid test. The extract was dissolved in ethanol and treated with 1 mL concentrated HCl. A colour change (yellow/orange/green) indicated flavonoids [12].

Saponin test. Distilled water was heated for 15 min, then 2 mL extract was added and shaken for 1 min to form foam. After standing for 5 min, one drop of HCl was added, and stable foam indicated saponins [13].

Terpenoid test. Two mL fraction was treated with 10 drops glacial CH_3COOH and 2 drops concentrated H_2SO_4, then allowed to stand. Red/purple colour indicated terpenoids [12].

Steroid test. Two mL fraction was treated with 10 drops glacial CH_3COOH and 2 drops concentrated H_2SO_4, then allowed to stand. Blue/green colour indicated steroids [14].

Preparation of Tris buffer saline and BSA solution

Tris buffer saline (TBS) was prepared by dissolving 4.35 g NaCl in 200 mL distilled water, adding 605 mg Tris buffer, adjusting pH with glacial acetic acid to pH 6.2–6.5, and completing the final volume as specified [15]. A 0.2% BSA solution was prepared by dissolving 0.2 g BSA and diluting to 100 mL using TBS [15].

Preparation of negative control, extract solutions, and positive control

The negative control consisted of 0.2% BSA with ethanol solvent adjusted to a total volume of 5 mL [15]. Stock solutions (2,000 ppm) of the *A. paniculata* leaf ethanolic extract and sodium diclofenac were prepared by dissolving 50 mg of each material in 25 mL ethanol. Working solutions were then prepared to obtain final assay concentrations of 5, 10, 15, 20, and 25 ppm. Sodium diclofenac served as a positive control reference NSAID [16].

BSA protein denaturation inhibition assay

A volume of 500 µL of each working solution (extract or diclofenac) at each concentration was mixed with 0.2% BSA and adjusted to 5 mL total volume to yield final concentrations of 5, 10, 15, 20, and 25 ppm. The mixtures were incubated at 37°C for 15 min, heated at 70°C for 5 min, cooled, and gently shaken prior to measurement. Absorbance was measured at 660 nm using a UV–Vis spectrophotometer, where the readout should be interpreted as denaturation-associated turbidity/scattering rather than an intrinsic protein absorbance maximum [7].

Calculation of percent inhibition and IC_{50}

Percent inhibition was calculated as:

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

where $A_{control}$is the absorbance of the negative control (BSA + ethanol) and $A_{sample}$is the absorbance of the test mixture. IC_{50} was estimated by regression analysis of percent inhibition versus concentration and interpolation at 50% inhibition.

Statistical analysis

Quantitative outcomes were summarised as mean ± SD from replicate measurements. Group comparisons were analysed using one-way ANOVA at α = 0.05 as specified in the manuscript [7].

3. Results and Discussion

Extraction Output and Yield of *Andrographis paniculata* Leaf Ethanolic Extract

The plant material used in this study comprised leaves of *Andrographis paniculata* collected from the sampling location described in the manuscript. The leaves were cleaned, dried to obtain simplicia, and milled into a homogeneous powder prior to extraction. Maceration was then conducted using 96% ethanol as the extraction solvent to obtain a concentrated ethanolic extract for subsequent phytochemical screening and BSA protein denaturation testing. This extraction approach is consistent with solvent-based extraction strategies that aim to recover a broad range of polar to semipolar constituents from botanical matrices, while also supporting downstream chemical characterisation such as fingerprinting and chromatographic profiling for quality-oriented interpretation [9].

Table 1. Extraction output and yield of *Andrographis paniculata* leaf ethanolic extract

Solvent	Solvent Volume (mL)	Dried Simplicia (g)	Concentrated Extract (g)	Yield (%)
Ethanol (96%)	2000	300	42	14

A total of 300 g dried leaf simplicia produced 42 g of concentrated extract, corresponding to a 14% yield (**Table 1**). Percentage yield is a practical indicator of extraction performance, representing the proportion of extractable material recovered from the starting plant matrix under defined solvent and process conditions. In the context of *in vitro* bioassays, a stable and adequately sized yield is methodologically important because it ensures sufficient extract mass for replicate concentration–response testing and improves the reproducibility of subsequent phytochemical and activity assessments. Moreover, the use of 96% ethanol plausibly supports the recovery of multiple secondary metabolite classes of differing polarity, thereby providing an extract that is chemically representative of the leaf matrix and suitable for initial activity screening, while remaining compatible with further analytical evaluation such as solvent-dependent fingerprint or chromatographic standardisation strategies [9].

Phytochemical Screening Profile of *A. paniculata* Leaf Ethanolic Extract

Qualitative phytochemical screening of the 96% ethanolic leaf extract of *Andrographis paniculata* showed the presence of alkaloids, flavonoids, tannins, saponins, and terpenoids, whereas steroids were not detected under the applied tube-test conditions (**Table 2**). The detection of these metabolite classes is pharmacologically plausible in the context of anti-inflammatory screening, because *A. paniculata* is widely recognised to contain bioactive constituents, including diterpenoid lactones such as andrographolide and other secondary metabolites that have been associated with inflammation-related modulation in the literature [2], [4]. In particular, flavonoids and terpenoid-related constituents are frequently discussed as candidate contributors to

anti-inflammatory effects through multiple biochemical interactions, although the present study does not resolve specific molecular identities or mechanistic targets beyond the proxy model employed.

Table 2. Phytochemical screening results of *Andrographis paniculata* leaf ethanolic extract

Compound Class	Reagent/Method	Observation (Positive Indicator)	Result
Alkaloids	Dragendorff/Mayer/Wagner tests [10].	Precipitate formation (reagent-dependent).	+
Flavonoids	Mg–HCl test [12].	Characteristic colour change.	+
Tannins	$FeCl_3$ test [11].	Dark green/blackish coloration.	+
Saponins	Froth test (hot water + HCl) [13].	Persistent foam after standing.	+
Terpenoids	CH_3COOH + H_2SO_4 (colour reaction) [12].	Red/purple coloration.	+
Steroids	CH_3COOH + H_2SO_4 (colour reaction) [14].	Blue/green coloration.	–

BSA Protein Denaturation Assay: Absorbance Pattern and Percent Inhibition

The *in vitro* anti-inflammatory activity of *Andrographis paniculata* leaf ethanolic extract was evaluated using the BSA protein denaturation inhibition model, in which inhibition is quantified by comparing the absorbance of the test mixture against the negative control (BSA + solvent, $A_{control}$). This assay is widely applied as an initial screening proxy for anti-inflammatory potential, because thermal denaturation increases solution turbidity and the corresponding spectrophotometric signal, while effective inhibitors reduce denaturation-associated turbidity under the same conditions [6],[8], [15],[18],[19]. Importantly, the readout at 660 nm should be interpreted as turbidity/denaturation-associated light scattering rather than an intrinsic "protein maximum absorbance" [6].

As shown in **Table 3**, the *A. paniculata* extract exhibited a clear concentration-dependent response across 5–25 ppm, where percent inhibition increased from 21.306% (5 ppm) to 72.821% (25 ppm). This pattern was accompanied by a progressive decrease in absorbance from 1.116 to 0.385, indicating stronger suppression of denaturation-induced turbidity at higher extract concentrations. In parallel, sodium diclofenac (positive control) demonstrated consistently higher inhibition at equivalent concentrations, increasing from 49.025% (5 ppm) to 83.462% (25 ppm), with lower absorbance values (0.723 to 0.234). This comparative profile is expected because diclofenac is a reference NSAID with established anti-inflammatory potency and is commonly used to benchmark inhibition strength in denaturation-based screening models [16]. Collectively, these results indicate that the *A. paniculata* leaf extract possesses measurable anti-denaturation activity within this screening framework, although the magnitude of inhibition remains lower than diclofenac at matched concentrations, supporting a proportional interpretation that the extract is a promising screening-level candidate rather than an equivalent comparator to the NSAID standard [6],[8].

Table 3. BSA protein denaturation inhibition activity of *Andrographis paniculata* leaf ethanolic extract and sodium diclofenac

Sample	Concentration (ppm)	Absorbance (660 nm)	% Inhibition
A. paniculata leaf ethanolic extract	5	1.116	21.306
A. paniculata leaf ethanolic extract	10	0.916	35.447
A. paniculata leaf ethanolic extract	15	0.724	48.931
A. paniculata leaf ethanolic extract	20	0.584	58.820
A. paniculata leaf ethanolic extract	25	0.385	72.821
Sodium diclofenac	5	0.723	49.025
Sodium diclofenac	10	0.664	53.182
Sodium diclofenac	15	0.544	61.639
Sodium diclofenac	20	0.307	78.341
Sodium diclofenac	25	0.234	83.462

Note: $A_{control}$represents the negative control (BSA + solvent). Based on the inhibition calculations, $A_{control}$was approximately 1.418 for this assay series.

Dose–Response Modelling, Regression Type, and IC_{50}

To quantify inhibitory strength and enable comparative interpretation, the concentration–response relationship was modelled and IC_{50} values were estimated for both the *Andrographis paniculata* leaf ethanolic extract and sodium diclofenac. Based on the regression equations presented, the fitted model for both datasets follows the form y = a ln(C) + b, where *y* is percent inhibition and *C* is concentration (ppm). Therefore, the appropriate interpretation is semi-log linear regression, not linear regression on raw concentration values. This distinction is methodologically important because it ensures consistency between the analytical model, the plotted regression (**Figure 1–2**), and the IC_{50} interpolation procedure, and it aligns with reporting practices in denaturation-based *in vitro* anti-inflammatory screening studies that frequently use regression-based interpolation for potency estimation [15], [17]–[19].

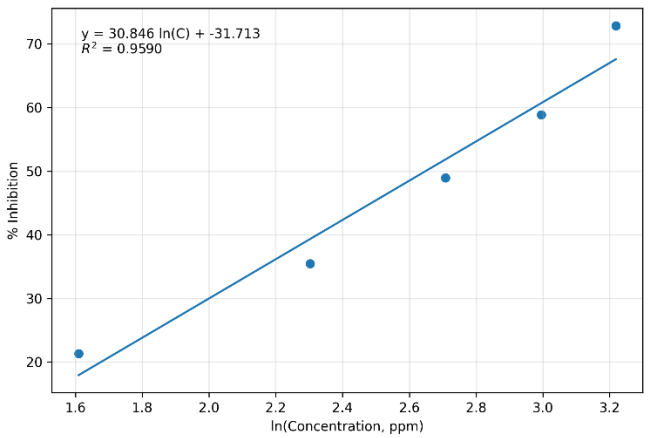

Figure 1. Semi-log linear regression of percent inhibition versus ln(concentration) for *A. paniculata* leaf ethanolic extract in the BSA protein denaturation assay

As shown in **Table 4,** the extract regression produced the equation y = 34.654 ln(C) − 34.550 with R^2 = 0.9628 (**Figure 1**), whereas diclofenac followed y = 21.952 ln(C) + 22.550 with R^2 = 0.9060 (**Figure 2**). The R^2 statistic indicates the degree of fit of the selected regression model to the observed data within the tested concentration range, but it should not be interpreted as a direct measure of biological effectiveness. The biologically interpretable potency metric is IC_{50}, defined as the concentration required to achieve 50% inhibition of protein denaturation under the assay conditions. Using regression-based interpolation at y = 50%, the IC_{50} of the *A. paniculata* leaf extract was 14.1467 ppm, while sodium diclofenac showed a lower IC_{50} of 4.9647 ppm (**Table 4**). This difference indicates that diclofenac reaches the 50% inhibition threshold at a substantially lower concentration, which is consistent with its established pharmacological potency as a reference NSAID in denaturation-based screening models [16],[18],[19].

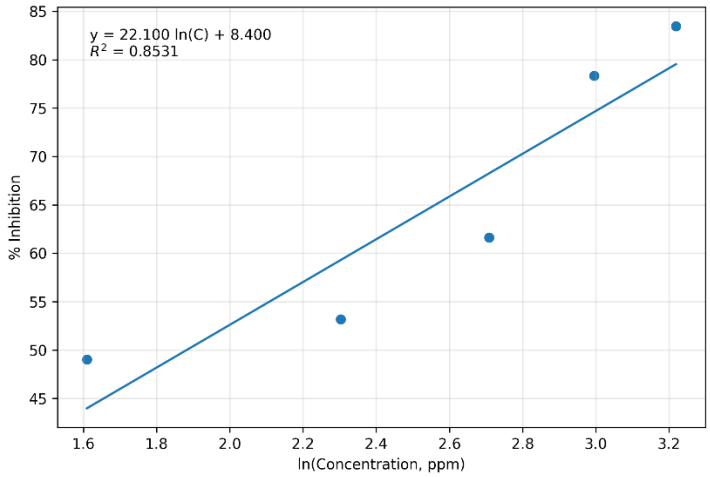

Figure 2. Semi-log linear regression of percent inhibition versus ln(concentration) for sodium diclofenac in the BSA protein denaturation assay.

Table 4. Semi-log regression equations, goodness-of-fit, and IC_{50} values in the BSA denaturation assay

Sample	**Regression equation (y = % inhibition; C in ppm)**	**R^2**	**IC_{50} (ppm)**
A. paniculata leaf ethanolic extract	y = 34.654 ln(C) − 34.550	0.9628	14.1467
Sodium diclofenac	y = 21.952 ln(C) + 22.550	0.9060	4.9647

Nevertheless, the extract's IC_{50} value in the low-ppm range supports the interpretation that the ethanolic leaf extract possesses measurable anti-denaturation activity within this proxy model and may warrant further investigation as a screening-level candidate. In the broader literature, crude botanical extracts commonly show improved inhibitory profiles after standardisation, fractionation, or enrichment of dominant bioactive constituents, and therefore the present IC_{50} should be positioned as an initial potency estimate rather than a definitive efficacy marker [15], [17]–[19]. In addition, prior work on *A. paniculata* has also combined *in vitro* screening with complementary computational approaches to explore molecular plausibility, suggesting that subsequent integration of standardised chemical profiling and confirmatory

mechanistic assays may strengthen translational interpretation beyond the present denaturation proxy [20].

Statistical Analysis and Interpretation

To support inferential interpretation of the spectrophotometric outcomes, a between-group comparison was conducted using one-way ANOVA, a statistical approach that is frequently reported in *in vitro* protein denaturation studies to evaluate whether the observed responses differ systematically across predefined experimental groups under comparable assay conditions [17],[18],[19]. In the current manuscript, the ANOVA summary presented in **Table 5** corresponds to the absorbance values at 660 nm (mean ± SD) for each group, rather than percent inhibition, because the reported means (0.858 and 0.495) fall on the absorbance scale and are not expressed in the 0–100% inhibition range.

Table 5. One-way ANOVA summary for group comparison based on absorbance (660 nm) in the BSA denaturation assay

Group	Mean ± SD (Absorbance)	p-value (between groups)
A. paniculata leaf ethanolic extract	0.858 ± 0.352	—
Sodium diclofenac	0.495 ± 0.199	0.001

Note: The negative control is defined as ***BSA + solvent*** *($A_{control}$). Lower absorbance indicates lower denaturation-associated turbidity and is consistent with higher inhibition.*

Given that higher denaturation yields higher turbidity and therefore higher absorbance, the lower mean absorbance observed for sodium diclofenac indicates stronger suppression of denaturation-associated turbidity relative to the *A. paniculata* extract, which is directionally consistent with the potency comparison shown in **Table 3**. The one-way ANOVA returned $p = 0.001$ (reportable as $p = 0.001$, or $p < 0.01$), indicating a statistically significant difference between the two groups under the analysed dataset. Importantly, the p-value reflects the difference produced by the analysed grouping strategy; therefore, if the scientific question is specifically dose-wise differences across concentrations, the more defensible approach is to run one-way ANOVA within each sample across concentration levels (with post-hoc testing), using % inhibition as the primary endpoint, because % inhibition directly represents anti-denaturation activity in this assay model [17],[18],[19].

Comparative Discussion and Mechanistic Plausibility

The present study demonstrates that the 96% ethanolic leaf extract of *Andrographis paniculata* exhibits measurable inhibition of heat-induced BSA denaturation, with a concentration-dependent increase in percent inhibition and an IC_{50} of 14.1467 ppm (**Table 3–4; Figure 1**). When positioned against the positive control, sodium diclofenac (IC_{50} 4.9647 ppm; **Figure 2**), the extract shows lower inhibitory potency within this proxy model, yet remains biologically interpretable as a screening-level candidate because crude extracts typically represent complex mixtures in which active constituents are diluted by non-active matrix components. This interpretation is consistent with the broader pharmacognosy literature that recognises *A. paniculata* as a medicinal plant rich in bioactive constituents, particularly diterpenoid lactones such as andrographolide, alongside flavonoids and other secondary metabolites that have been repeatedly linked to pharmacological activity, including anti-inflammatory effects [2], [4].

In the present phytochemical dataset, the detection of flavonoids, tannins, saponins, and terpenoids provides a plausible chemical context for the observed anti-denaturation activity, because these classes are frequently associated with protein-interactive and redox-modulatory behaviours that may contribute to stabilisation of protein conformation under stress conditions. Nevertheless, the mechanistic interpretation must remain cautious: the BSA denaturation assay is fundamentally a protein-stabilisation proxy and does not directly demonstrate inhibition of canonical inflammatory enzymatic pathways (e.g., COX) or intracellular signalling networks. Therefore, mechanistic statements should be framed as plausibility rather than proof. In this context, andrographolide has been discussed in the literature as a candidate molecule with the potential to modulate inflammatory signalling, including plausible interaction with NF-κB–related pathways, and computational or molecular-based analyses have been used to support such mechanistic hypotheses at the target-interaction level [5]. Moreover, prior studies on *A. paniculata* have combined *in vitro* screening with complementary *in silico* approaches to strengthen biological plausibility and prioritise candidate constituents for further validation, indicating a rational direction for subsequent work that integrates extract standardisation and confirmatory mechanistic assays beyond the present denaturation proxy [20].

Study limitations

This study has several limitations that should be considered when interpreting the findings. The BSA protein denaturation assay is an *in vitro* proxy that reflects suppression of thermally induced protein structural disruption and associated turbidity, but it does not directly confirm anti-inflammatory efficacy in biological systems nor identify specific molecular targets. In addition, the phytochemical screening was qualitative and limited to presence/absence classification of broad metabolite groups, so the active constituents and their relative contributions cannot be determined from the current dataset. The use of a crude ethanolic extract also means that the observed potency may be influenced by matrix effects and assay conditions, while the regression-derived IC_{50} is dependent on the tested concentration range and the selected model. Accordingly, future investigations should prioritise extract standardisation (e.g., quantitative markers and chromatographic fingerprinting), fractionation or enrichment to identify dominant active fractions, and confirmatory assays that interrogate more specific inflammatory mechanisms, followed by validation in cellular or in vivo models to strengthen translational relevance.

4. Conclusions

The 96% ethanolic leaf extract of *Andrographis paniculata* demonstrated the presence of alkaloids, flavonoids, tannins, saponins, and terpenoids based on qualitative phytochemical screening, while steroids were not detected. In the BSA protein denaturation inhibition assay, the extract exhibited a clear concentration-dependent increase in inhibition across 5–25 ppm, rising from 21.306% to 72.821%, and produced an IC_{50} of 14.1467 ppm, indicating measurable anti-denaturation activity within this *in vitro* screening model. Sodium diclofenac showed stronger inhibitory potency with a lower IC_{50} of 4.9647 ppm, confirming its expected role as a reference NSAID comparator. Overall, these findings support that *A. paniculata* leaf ethanolic extract possesses *in vitro* anti-inflammatory potential in a protein-stabilisation proxy assay and warrants further standardisation and confirmatory testing using more mechanistically informative models.

Acknowledgements:
The authors gratefully acknowledge the Department of Pharmacy, Gorontalo State University, Indonesia, for providing laboratory facilities and institutional support for this study. Technical assistance from laboratory staff during sample preparation and experimental procedures is also sincerely appreciated.

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

References

[1] N. Putri, L. Listyalina, B. F. Wahidah, *et al.*, "Antibacterial activity of *Andrographis paniculata* (Nees) ethanol extract against *Staphylococcus*," 2023, pp. 1–9. [Online]. Available: https://doi.org/10.58533/852kk888

[2] T. Jayakumar, C.-H. Hsieh, J.-J. Lee, and J.-R. Sheu, "Experimental and clinical pharmacology of *Andrographis paniculata* and its major bioactive phytoconstituent andrographolide," *Evidence-Based Complementary and Alternative Medicine*, 2013. [Online]. Available: https://doi.org/10.1155/2013/846740

[3] T. Sirikarin, T. Palo, S. Chotewuttakorn, W. Chandranipapongse, S. Limsuvan, and P. Akarasereenont, "The effects of *Andrographis paniculata* on platelet activity in healthy Thai volunteers," *Evidence-Based Complementary and Alternative Medicine*, 2018. [Online]. Available: https://doi.org/10.1155/2018/2458281

[4] K. Jarukamjorn and N. Nemoto, "Pharmacological aspects of *Andrographis paniculata* on health and its major diterpenoid constituent," *J. Health Sci.*, vol. 54, pp. 370–381, 2008. [Online]. Available: https://doi.org/10.1248/jhs.54.370

[5] P. J. C. Sudandiradoss, "Andrographolide-based potential anti-inflammatory transcription inhibitors against nuclear factor NF-κB p50 subunit: An integrated molecular and quantum mechanical approach," *3 Biotech*, vol. 13, pp. 1–19, 2023. [Online]. Available: https://doi.org/10.1007/s13205-022-03431-9

[6] A. R. Nirmala, L. Permatasari, H. Muliasari, and R. F. Deccati, "Review: Optimization of bovine serum albumin (BSA) protein-denaturation inhibition method for anti-inflammatory activity testing across various leaf extracts," 2023. [Online]. Available: https://doi.org/10.31764/jafp.v3i2.20953

[7] M. Hdt and S. Dnaw, "In vitro anti-inflammatory egg albumin denaturation assay: An enhanced approach," 2023, pp. 1–6. [Online]. Available: https://doi.org/10.23880/jonam-16000411

[8] N. I. Osman, N. J. Sidik, A. Awal, and T. Mara, "In vitro xanthine oxidase and albumin denaturation inhibition assay of *Barringtonia racemosa* L. and total phenolic content analysis for potential anti-inflammatory use in gouty arthritis," 2016. [Online]. Available: https://doi.org/10.5455/jice.20160731025522

[9] R. Notes, M. Rafi, A. F. Devi, U. D. Syafitri, R. Heryanto, I. H. Suparto, *et al.*, "Classification of *Andrographis paniculata* extracts by solvent extraction using HPLC fingerprint and chemometric analysis," *BMC Res. Notes*, 2020. [Online]. Available: https://doi.org/10.1186/s13104-020-4920-x

[10] H. Hasan, E. N. Djuwarno, H. Samudi, and W. Susanti, "Antidiabetic compounds in the active fraction of ketapang (*Terminalia catappa* L.) leaves," *J. Syifa Sci. Clin. Res.*, vol. 4, pp. 517–529, 2022. [Online]. Available: https://repository.ung.ac.id/en/skripsi/show/821418014/senyawa-antidiabetes-fraksi-aktif-daun-ketapang-terminalia-catappa-l.html

[11] A. S. Abubakar, X. Huang, Z. M. Birhanie, G. Gao, and X. Feng, "Phytochemical composition, antioxidant, antibacterial, and enzyme inhibitory activities of

various organic extracts from *Apocynum hendersonii* (Hook.f.) Woodson," *Plants*, 2022. [Online]. Available: https://doi.org/10.3390/plants11151964

[12] R. Ciencia, I. Elect, L. Andes, *et al.*, "Phytochemical screening of *Jatropha curcas* L. leaves and roots collected from Mérida, Venezuela," 2020, vol. 41, pp. 75–80. [Online]. Available: https://www.scispace.com/pdf/phytochemical-screening-of-jatropha-curcas-l-leaves-and-1lyaf4138k.pdf

[13] D. A. Setiabudi and Tukiran, "Phytochemical screening of methanolic extract of klampok watu (*Syzygium litorale*) stem bark," *UNESA J. Chem.*, vol. 6, pp. 156–160, 2017. [Online]. Available: https://123dok.com/document/zgw1rko8-skrining-fitokimia-ekstrak-metanol-tumbuhan-klampok-syzygium-litorale.html

[14] S. M. Astuti, "Determination of saponin compound from *Anredera cordifolia* (Ten.) Steenis (binahong) to potential treatment for several diseases," *J. Agric. Sci.*, vol. 3, pp. 224–232, 2011. [Online]. Available: https://doi.org/10.5539/jas.v3n4p224

[15] S. Warnasih, U. Hasanah, S. J. Simalango, A. H. Mulyati, and D. Widiastuti, "In-vitro and in-silico study: The anti-inflammatory activity of ethanol extract from cogon grass roots (*Imperata cylindrica* L.)," 2025, pp. 264–276. [Online]. Available: https://doi.org/10.20473/jfiki.v12i22025

[16] R. Altman, B. Bosch, K. Brune, P. Patrignani, and C. Young, "Advances in NSAID development: Evolution of diclofenac products using pharmaceutical technology," *Drugs*, vol. 75, pp. 859–877, 2015. [Online]. Available: https://doi.org/10.1007/s40265-015-0392-z

[17] P. Sherly, A. Santianingtyas, M. Dzakwan, and F. Leviana, "In vitro analysis of anti-inflammatory activity of lyotropic liquid crystal nanoparticles from *Echinacea purpurea* flower extract," 2024, pp. 356–378. [Online]. Available: https://doi.org/10.35311/jmpi.v10i2.555

[18] A. Fristiohady, I. Sahidin, B. Sadarun, L. O. M. J. Purnama, and S. Nur, "Anti-inflammatory activity of ethanolic extract of *Melophlus* sp. and *Callyspongia* sp. from Southeast Sulawesi," *Indonesian J. Pharm. Sci. Technol.*, 2024. [Online]. Available: https://doi.org/10.24198/ijpst.v11i1.28809

[19] A. P. Patel, S. P. Dhavan, and T. R. Shinde, "Evaluation of in vitro anti-inflammatory activity of *Trichosanthes palmata* against the denaturation of protein," 2025, pp. 1–5. [Online]. Available: https://doi.org/10.5530/pres.20252089

[20] S. Samajdar, I. Mondal, and P. Roy, "Anti-inflammatory evaluation of *Andrographis paniculata* leaf extract formulation using a combination of in silico and in vitro methods," 2023, vol. 23, pp. 1301–1307. [Online]. Available: https://ymerdigital.com/uploads/YMER2310E9.pdf