Artículo Original

Volumen 16, N° 33. Julio-Diciembre 2026
Thiyagarajan y Col.
Pg. 38-61

DOI:

Acta BIOCLÍNICA
ISSN Electrónico: 3105-403X

EVALUATION OF IN VITRO ANTI-INFLAMMATORY ACTIVITY OF *SIDA ACUTA*

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ABSTRACT

BACKGROUND & OBJECTIVES: Non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are the most commonly used anti-inflammatory drugs in clinical practice, despite their side effects [1,2] . This indicates the need for the development of an effective, safe, and economical anti-inflammatory drug with fewer harmful side effects. In many developing countries, plants used in medicine are the main source of therapy, but only a few have strong scientific backing [3]. Sida acuta, also known as the common wireweed, is a pan-tropical shrub

Received: 20-03-2026
Accepted: 30-04-2026

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DOI:

Acta BIOCLÍNICA
ISSN Electrónico: 3105-403X

used in different herbal medical practices. Recent studies have shown its significant anti-inflammatory activity compared to other conventional anti-inflammatory drugs. The aim of this study is to determine the in vitro anti-inflammatory activity of Sida acuta, to confirm its traditional use in the treatment of inflammation, and to explain its mechanism of action. MATERIALS & METHODS: The anti-inflammatory activity of Sida acuta was determined by comparing it with the standard diclofenac sodium using two different methods [4,5]. 1) Hypotonicity-induced human red blood cell (HRBC) membrane stabilization assay: Samples of 200 to 1000 µg, prepared in phosphate buffer solution, were tested against diclofenac sodium as a standard. The extent of HRBC hemolysis and membrane stabilization (protection) was determined spectrophotometrically by measuring the optical density at 560 nm. The anti-inflammatory activity was measured as the percentage of RBC lysis. 2) Bovine Serum Albumin (BSA) Denaturation Assay, where ethanol extracts of the sample (200-1000 µg concentrations) were compared with standard aspirin as a control. The level of protein precipitation was measured in terms of optical density (OD) spectrophotometrically at 660 nm. Anti-inflammatory activity is expressed as the percentage of protein denaturation % of inhibition of hemolysis or protein denaturation (anti-inflammatory activity) = (O.D. of Control - O.D. of Sample) / (O.D. of Control) X 100. RESULT: The sample extracts are able to stabilize the erythrocyte membrane in hypotonic solution and exhibit more major activity than diclofenac sodium at different doses (200–1000 µg concentrations). The HRBC membrane acts similarly to the lysosomal membrane. If HRBC membrane stabilizes by using the extract, then it means the extracts also stabilizes the lysosomal membrane. The sample also demonstrated a very high percentage of inhibition of

Received: 20-03-2026
Accepted: 30-04-2026

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Acta BIOCLÍNICA
ISSN Electrónico: 3105-403X

protein denaturation compared to aspirin, thereby exhibiting its higher anti-inflammatory potency. CONCLUSION: In this study, we showed that the Sida acuta extracts have enormous potential and can be used to inhibit the inflammatory responses. These findings collectively underscore the therapeutic potential of Sida acuta as an anti-inflammatory agent, validating its traditional applications and suggesting avenues for further research and development in phytomedicine.

KEYWORDS: *Sida acuta*; Anti-inflammatory; HRBC; Albumin denaturation; protein denaturation; Medicinal plant; Diclofenac; In vitro assays.

EVALUACIÓN DE LA ACTIVIDAD ANTIINFLAMATORIA IN VITRO DE *SIDA ACUTA*

RESUMEN

ANTECEDENTES Y OBJETIVOS: Los antiinflamatorios no esteroideos (AINE) y los corticosteroides son los antiinflamatorios más utilizados en la práctica clínica, a pesar de sus efectos secundarios [1,2]. Esto indica la necesidad de desarrollar un antiinflamatorio eficaz, seguro y económico con menos efectos secundarios perjudiciales. En muchos países en desarrollo, las plantas utilizadas en medicina son la principal fuente de terapia, pero solo unas pocas cuentan con un sólido respaldo científico [3]. Sida acuta, también conocida como hierba de alambre común, es un arbusto pantropical utilizado en diversas prácticas de medicina herbal. Estudios recientes han demostrado su importante actividad antiinflamatoria en comparación con otros

Received: 20-03-2026
Accepted: 30-04-2026

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ISSN Electrónico: 3105-403X

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antiinflamatorios convencionales. El objetivo de este estudio es determinar la actividad antiinflamatoria in vitro de Sida acuta, confirmar su uso tradicional en el tratamiento de la inflamación y explicar su mecanismo de acción. MATERIALES Y MÉTODOS: La actividad antiinflamatoria de Sida acuta se determinó comparándola con el diclofenaco sódico estándar utilizando dos métodos diferentes [4,5]. 1) Ensayo de estabilización de la membrana de eritrocitos humanos (HRBC) inducido por hipotonicidad: Se probaron muestras de 200 a 1000 µg, preparadas en solución tampón de fosfato, frente al diclofenaco sódico como estándar. El grado de hemólisis de HRBC y estabilización (protección) de la membrana se determinó espectrofotométricamente midiendo la densidad óptica a 560 nm. La actividad antiinflamatoria se midió como el porcentaje de lisis de eritrocitos. 2) Ensayo de desnaturalización de albúmina sérica bovina (BSA), donde los extractos de etanol de la muestra (concentraciones de 200-1000 µg) se compararon con aspirina estándar como control. El nivel de precipitación de proteínas se midió en términos de densidad óptica (DO) espectrofotométricamente a 660 nm. La actividad antiinflamatoria se expresa como el porcentaje de desnaturalización de proteínas % de inhibición de la hemólisis o desnaturalización de proteínas (actividad antiinflamatoria) = (D.O. del control - D.O. de la muestra) / (D.O. del control) X 100. RESULTADO: Los extractos de la muestra son capaces de estabilizar la membrana del eritrocito en solución hipotónica y exhiben una actividad mayor que el diclofenaco sódico en diferentes dosis (concentraciones de 200 a 1000 µg). La membrana de los eritrocitos humanos actúa de forma similar a la membrana lisosomal. Si la membrana de los eritrocitos humanos se estabiliza con el uso del extracto, entonces significa que el extracto también estabiliza la membrana lisosomal. La muestra también demostró un

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Accepted: 30-04-2026

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porcentaje muy alto de inhibición de la desnaturalización de proteínas en comparación con la aspirina, lo que demuestra su mayor potencia antiinflamatoria. CONCLUSIÓN: En este estudio, demostramos que los extractos de Sida acuta tienen un enorme potencial y pueden utilizarse para inhibir las respuestas inflamatorias. Estos hallazgos, en conjunto, subrayan el potencial terapéutico de Sida acuta como agente antiinflamatorio, validando sus aplicaciones tradicionales y sugiriendo vías para futuras investigaciones y desarrollos en fitomedicina.

PALABRAS CLAVE: learning Sida acuta; Antiinflamatorio; HRBC; Desnaturalización de albúmina; Desnaturalización de proteínas; Planta medicinal; Diclofenaco; Ensayos in vitro.

INTRODUCTION

Inflammation is a basic protective mechanism of the body in response to tissue injury, infection, or injurious stimuli, with the goal of eliminating the underlying cause of cellular injury and initiating the repair of damaged tissues [1,6] .The process of inflammation involves a complex cascade of cellular and biochemical reactions mediated by inflammatory cells, cytokines, prostaglandins, and lysosomal enzymes. Although acute inflammation is physiological and self-limiting, chronic inflammatory conditions can arise from persistent and uncontrolled inflammation, which plays a significant role in the pathogenesis of various diseases, including rheumatoid arthritis, osteoarthritis, cardiovascular diseases, neurodegenerative disorders, metabolic syndrome, and certain cancers [1] .

Received: 20-03-2026
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Non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are the cornerstone of anti-inflammatory therapy. These drugs have been found to be effective in the treatment of inflammation by primarily inhibiting cyclooxygenase enzymes and reducing the synthesis of inflammatory mediators [2,9]. However, the chronic use of NSAIDs has been found to be associated with severe side effects, including gastrointestinal ulceration, renal dysfunction, cardiovascular complications, and hypersensitivity reactions. Similarly, the chronic administration of corticosteroids has been found to be associated with immunosuppression, osteoporosis, metabolic disorders, and adrenal insufficiency. These drawbacks make it imperative to look for safer, more effective, and more economical alternatives, especially from natural sources [2,3].

Medicinal plants have long been used in traditional systems of medicine and are a rich source of bioactive compounds with therapeutic potential [3,10]. In many developing nations, herbal medicines have continued to be the mainstay of healthcare due to their ease of availability, affordability, and perceived safety. However, despite their widespread use in traditional medicine, scientific proof of many medicinal plants is still limited [4,5].

Sida acuta , of the family Malvaceae, is a pantropical shrub known as common wireweed. It has long been used in traditional folk medicine for the treatment of pain, inflammation, fever, wounds, and infectious diseases [4,11]. Phytochemical

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analysis of Sida acuta has shown the presence of flavonoids, alkaloids, phenolic compounds, saponins, and glycosides, many of which have been found to possess antioxidant and anti-inflammatory properties [12-14] . Earlier studies have indicated that Sida acuta extracts may possess membrane-stabilizing and protein-protective properties, which are vital in the inhibition of inflammatory responses [6-9].

There are many in vitro techniques that are used for testing the potential of compounds to have an anti-inflammatory effect. Some of these techniques include assays that test the ability of a compound to stabilize human red blood cell (HRBC) membranes and to denature bovine serum albumin (BSA). The HRBC membrane has a similar structure to the lysosomal membrane, so when a compound can stabilize the HRBC membrane, then it indicates that this compound may have the ability to inhibit the release of lysosomal enzymes at the site of inflammation [15, 16]. Similarly, there is extensive documentation of protein denaturation being a major contributor to inflammation and tissue damage, so it is thought that agents that can inhibit protein denaturation will be effective as anti-inflammatory agents. [17]

Based on this background, the research aims to evaluate the in vitro anti-inflammatory property of the ethanolic extract of Sida acuta leaves. The research will make use of two validated assays: the HRBC membrane stabilization test and the BSA denaturation test, and compare the findings with the standard anti-

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inflammatory compounds. The research aims to scientifically prove the traditional claim of Sida acuta and to reveal the mechanism of its anti-inflammatory properties.

2. MATERIALS AND METHODS

2.1 Study Design

This in vitro study investigates the anti-inflammatory properties of Sida acuta leaf extracts using the HRBC membrane stabilization test[15,18] and the BSA denaturation test[17,19], which are well-established methods for evaluating anti-inflammatory activity. Diclofenac is used as the control to compare the effectiveness of the Sida acuta extract.

2.2 Selecting Plant Material and Authentication

The fresh leaves of Sida acuta were harvested from a local plant source. The plants were authenticated by a certified botanist to ensure that they were free from any diseases and contaminants before harvesting. After harvesting, the leaves were properly washed with distilled water to remove any dirt and debris and then shade-dried at room temperature for 7-10 days. After drying, the leaves were coarsely ground using a mechanical grinder and then packed in tightly sealed glass containers until the time of extraction. [6-9]

2.3 Extraction with Ethanol

The dried Sida acuta leaf powder was used to prepare crude ethanolic

Received: 20-03-2026
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extracts through maceration. The solvent used was a 70% ethanol solution, and the dried leaf material was submerged for 72 hours with occasional agitation to ensure the best possible extraction of the bioactive compounds. After maceration, the mixture was filtered using Whatman No. 1 filter paper to separate the plant material. The filtrate was then evaporated under reduced pressure using a rotary evaporator to obtain an amorphous semi-solid crude ethanolic extract, which was stored in a refrigerator at 4°C until further use. A set of diluted solutions of the crude extract was made by taking an aliquot and adding more ethanol to make a final concentration of 200, 400, 600, 800, and 1000 µg/ml.

2.4 Chemical Reagents and Other Materials

For this study, analytical-grade reagents were used. Diclofenac sodium was used as a control for anti-inflammatory properties. For other solutions, such as PBS, BSA, TBS, hyposaline, and the like, laboratory procedures were followed. Distilled water was used in all experiments and dilutions.

2.5 Preparation of Human Red Blood Cells (HRBCs)

2.5.1 Preparation of HRBCs [15,18]

Prior to blood collection, consent from a healthy donor was sought. Blood was collected in heparinized tubes and centrifuged at 3000 RPM for 10 minutes. The packed cells were then washed three times with isotonic PBS (pH 7.4). The final

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washed cells were re-suspended to a 10% v/v HRBC suspension in PBS.

2.5.2 Experimental Procedure

The hypotonicity-induced hemolysis method was used to evaluate the samples. Each reaction mixture contained:

- 1.0 mL of test sample at different concentrations (200-1000 µg/mL)
- 1.0 mL of 0.2 M phosphate buffer
- 0.5 mL of a 10% HRBC suspension
- 0.5 mL of 0.25% hyposaline solution

The mixtures were maintained at 37°C for 30 minutes, followed by centrifugation at 3,000 rpm for 20 minutes. Hemoglobin released into the supernatant was quantified as OD (Optical Density) using spectrophotometry at 560 nm.

Diclofenac sodium was used as the standard drug.

2.5.3 Calculation of Percentage Hemolysis

The percentage hemolysis was calculated using the formula:

% Hemolysis = [(OD of control − OD of test sample) / OD of control] × 100%

The extent of membrane stabilization was deduced from the extent to which the sample inhibited hemolysis compared to the control.

Received: 20-03-2026
Accepted: 30-04-2026

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2.6 Bovine Serum Albumin (BSA) Denaturation Assay

2.6.1 Preparation of BSA Solution [17,19]

A 0.4% (w/v) BSA solution is prepared in Tris-buffered saline (0.05 M Tris, 0.15 M NaCl, pH 7.6). The pH is then adjusted to 6.4 with glacial acetic acid to facilitate denaturation upon heating.

2.6.2 Experimental Procedure

In test tubes, 1 mL of the above-prepared BSA solution is mixed with Sida acuta extract at concentrations of 200, 400, 600, 800, and 1000 µg/mL. Diclofenac was used as the control. The mixtures are then incubated at 37°C for 20 minutes, followed by heating at 72°C for 10 minutes in a water bath to facilitate protein denaturation. The mixture is then cooled to room temperature for 20 minutes. Turbidity, which indicates protein precipitation, is measured at 660 nm using a UV-visible spectrophotometer. Quantification was done in terms of optical density (OD). Each experiment is performed in duplicate, and the mean value of the absorbance is recorded.

2.6.3 Calculation of Percentage Inhibition of Protein Denaturation

The percentage inhibition is calculated using the following formula:

% Inhibition = [(OD of control − OD of test sample) / OD of control] × 100%

The extent of protein denaturation was deduced from the extent to which the

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Accepted: 30-04-2026

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sample inhibited protein denaturation compared to the control.

2.7 Statistical Analysis

Results are expressed as mean ± SD (Standard Deviation). The density-dependent response and IC50 (half maximal inhibitory concentration) values were analyzed using linear regression. When comparing the test samples to the standard drugs, the relevant statistical tests were applied, and differences were considered significant if $p < 0.05$.

3. RESULTS

3.1 Human Red Blood Cell (HRBC) Membrane Stabilization Assay

For anti-inflammatory activity screening, the ethanolic extract of Sida acuta was evaluated for its membrane-stabilizing activity against hemolysis of red blood cells induced by hypotonic stress. The extract showed a concentration-dependent membrane-stabilizing activity in the range of 200-1000 µg/mL.

In the lower concentration range (200 µg/mL), there was a slight inhibition of hemolysis, suggesting partial membrane stabilization of RBCs. With increasing concentrations, hemolysis was inhibited significantly, with maximum membrane stabilization at 1000 µg/mL.[15,16]

A comparison between the Sida acuta extract (SAE) and the conventional NSAID, Diclofenac Sodium (DS), reveals significant differences in their ability to

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Accepted: 30-04-2026

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ISSN Electrónico: 3105-403X

protect red blood cell membranes from damage and lysis at all concentrations. The IC50 value of the Sida acuta extract is 1006.62 µg/mL, which is significantly lower than that of diclofenac sodium (4742.30 µg/mL), indicating that the Sida acuta extract is more effective and potent than the conventional NSAID in protecting erythrocytes from lysis under hyperosmotic stress.

Statistical analysis of the data has confirmed that the inhibition of red blood cell hemolysis by the Sida acuta extract, compared to the control, is significant ($P < 0.05$) and more effective. Linear regression analysis also indicates a good dose-response relationship, which further supports the therapeutic use of the Sida acuta extract for membrane stabilization.

Received: 20-03-2026
Accepted: 30-04-2026

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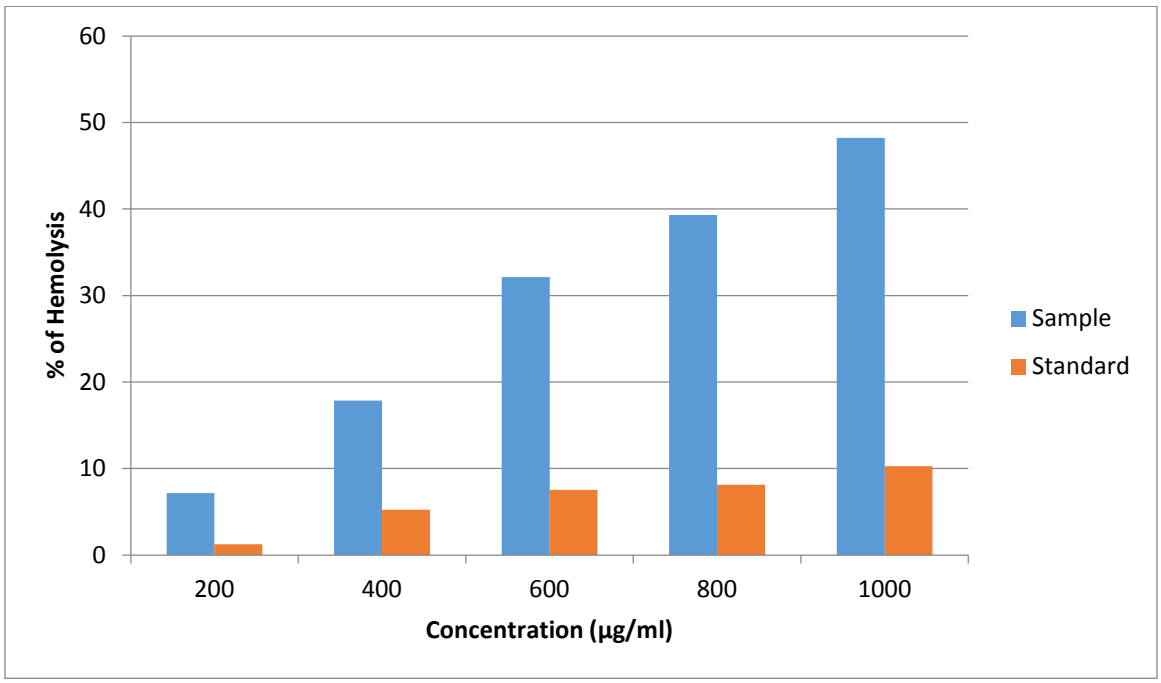
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Concentration (µg/ml)	OD Value	% of Hemolysis	
		Sample	Standard
200	0.52	7.142857	1.2540
400	0.46	17.85714	5.2369
600	0.38	32.14286	7.5142
800	0.34	39.28571	8.1246
1000	0.29	48.21429	10.2654

IC50 Value - 1006.6197 µg/ml (Sample) 4742.2952 µg/ml – Diclofenac



Received: 20-03-2026
Accepted: 30-04-2026

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3.2 Bovine Serum Albumin Denaturation Assay

The ethanol extract of Sida acuta was tested for its ability to inhibit heat-induced protein denaturation in this study using a bovine serum albumin denaturation assay. The ethanol extract was found to protect BSA from denaturation in a dose-dependent manner, suggesting that Sida acuta may have anti-inflammatory activities [17, 19]

At a concentration of 200 µg/mL, there was a slight inhibition of denaturation, and as the concentration increased, the inhibition increased proportionally with the concentration, reaching a maximum of 1000 µg/mL. The maximum inhibition of BSA denaturation was 53.13% at the maximum concentration of the extract, and this was significantly higher than that of the standard drug.

The Sida acuta extract had an IC50 value of 965.49 µg/mL, which is significantly lower than that of the standard drug of 3975.41 µg/mL, and this shows that Sida acuta is more potent as an inhibitor of protein denaturation.

At all concentrations, the inhibition of denaturation by the extract was significantly higher than the standard drug with $p < 0.05$. The results indicate a positive dose-response relationship, where a higher concentration of the extract indicates a higher percentage of inhibition of denaturation.

Received: 20-03-2026
Accepted: 30-04-2026

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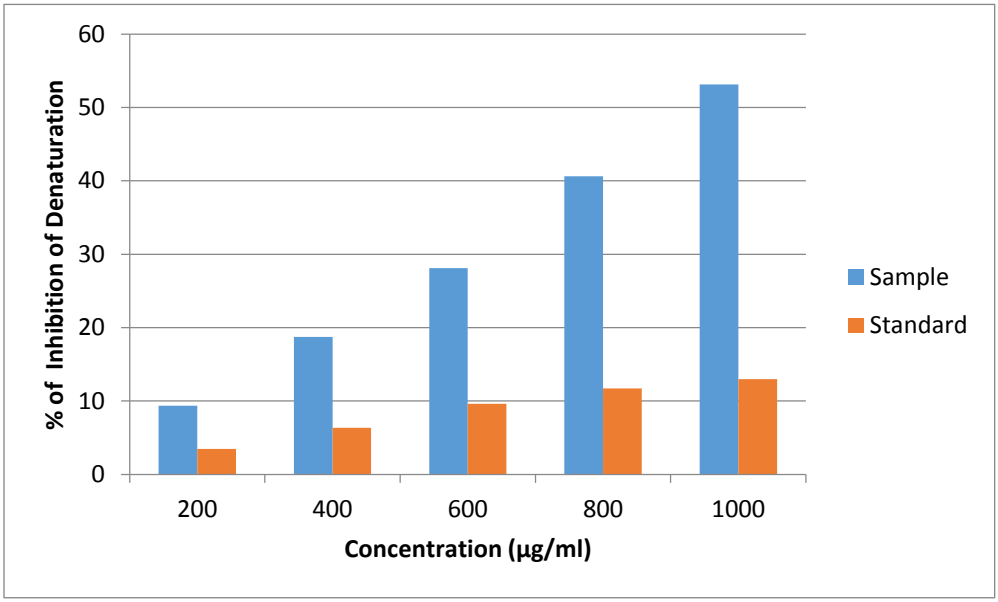
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Concentration (µg/ml)	Mean OD Value	% of Inhibitory Activity	
		Sample	Standard
200	0.29	9.375	3.454545
400	0.26	18.75	6.36364
600	0.23	28.125	9.63636
800	0.19	40.625	11.72727
1000	0.15	53.125	13

IC50 Value - 965.4936 µg/ml (sample) Standard – Diclofenac – 3975.41 µg/ml



Received: 20-03-2026
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3.3 Dose Response and Comparative Analysis

Collectively, the Sida acuta ethanolic extract shows a strong anti-inflammatory response in both protocols. The IC50 values of Sida acuta are lower than those of the standards in both protocols, suggesting that Sida acuta may be more potent and effective than the standard.

Both protocols display concentration-dependent responses, suggesting that the bioactive compounds in Sida acuta interact pharmacologically and in a predictable manner. The results are statistically significant at all concentrations tested, emphasizing that the observed responses are consistent in both protocols.

In conclusion, Sida acuta appears to strongly stabilize membranes and protect proteins, thus further supporting its anti-inflammatory properties by inhibiting the inflammatory process.

4. DISCUSSIONS

The current study offers strong in vitro evidence for the anti-inflammatory potential of the ethanolic extract of Sida acuta, as evidenced by HRBC membrane stabilization and BSA denaturation assays. Both experimental models are well-recognized preliminary screening procedures for the assessment of anti-inflammatory activity and provide valuable information regarding membrane protection and protein stabilization, which

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are key processes involved in the inflammatory response[1–3].

In the HRBC membrane stabilization assay, the Sida acuta extract showed a strong concentration-dependent inhibition of hypotonicity-induced hemolysis, with enhanced potency relative to diclofenac sodium. Membrane stabilization is a clear indicator of the potential of a compound to stabilize lysosomal membranes, thus preventing the release of proteolytic enzymes such as phospholipase A_2, elastase, and cathepsins at the site of inflammation[4–6]. This particular mechanism is highly relevant, given the pivotal role of lysosomal enzyme leakage in the exaggeration of tissue injury and inflammatory responses. This membrane-stabilizing activity has also been observed in Sida acuta leaf extracts by Oyedapo et al., who showed that membrane stabilization is the primary mechanism underlying the anti-inflammatory activity of Sida acuta.[7]

Similar results have also been obtained with other medicinal plants tested for their anti-inflammatory activity using HRBC models, such as Jatropha gossypifolia, Cardiospermum halicacabum, and Albuca setosa, thus establishing the importance of this assay[8–10].

The lower IC_{50} value obtained for the Sida acuta extract compared to diclofenac indicates its higher potency as a membrane protectant agent in an in vitro setup. This result is in line with previous studies suggesting that plant polyphenols and flavonoids have the potential to protect membranes in a manner comparable to or

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even better than conventional NSAIDs[11,12]. Unlike conventional NSAIDs, which are known to work by inhibiting cyclooxygenase enzymes and are linked to gastrointestinal and renal side effects, plant-derived compounds may offer anti-inflammatory properties by targeting multiple pathways with potentially improved safety profiles[13,14].

The BSA protein denaturation assay further reinforced the anti-inflammatory properties of Sida acuta. Protein denaturation is a known pathological process in inflammatory and autoimmune diseases such as rheumatoid arthritis, where denatured proteins can serve as neo-antigens and contribute to the chronic inflammatory process[15,16]. In the current investigation, the extract showed potent inhibition of heat-induced protein denaturation in a dose-dependent manner and proved to be more effective than the standard drug.

Similar inhibitory activities against protein denaturation have also been observed in other medicinal plants with high phenolic and flavonoid content, such as Oxalis corniculata, Plumeria acuminata, and certain Jamaican medicinal plants[17–19].

The anti-denaturation activity of Sida acuta can be attributed to its phytochemical constituents. Previous studies have shown the presence of flavonoids, alkaloids, tannins, saponins, and phenolic compounds in the leaves of Sida acuta[20]. Flavonoids have been extensively documented to possess anti-inflammatory properties by inhibiting the synthesis of

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inflammatory mediators, protecting proteins against denaturation, and scavenging reactive oxygen species, thus protecting tissues against oxidative stress-induced damage[21,22]. The role of phenolic compounds in further stabilizing membranes against lipid peroxidation and maintaining membrane integrity is also closely associated with decreased inflammatory signaling[23].

The observed dose-response consistency in both experimental models reinforces the biological significance of the results. The concomitant decrease in hemolysis and protein denaturation indicates that Sida acuta may exert its anti-inflammatory properties through a multi-mechanistic approach, which may include membrane stabilization, protein protection, and possibly antioxidant properties. The multi-mechanistic approach has been long recognized as a desirable characteristic of plant-based drugs over synthetic drugs that target a single mechanism[24,25].

However, although the in vitro findings are encouraging, they are still in the preliminary phase of pharmacological validation. In vivo inflammatory reactions are more complex, with interactions between immune cells, cytokines, transcription factors like NF-κB, and enzymatic pathways like cyclooxygenase and lipoxygenase pathways[11,15,19]. Thus, it is important to conduct further research using in vivo models of inflammation to validate the efficacy and safety of Sida acuta. Moreover, purification and characterization of the active compounds,

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followed by molecular biology studies, would also be helpful in understanding the specific targets and would aid in the development of standardized phytopharmaceuticals.

CONCLUSION:

The above study offers enough evidence to support the claim that the ethanolic extract of Sida acuta has strong anti-inflammatory properties in vitro. The extract showed the capacity to stabilize the human red blood cell membrane, thus suggesting that it has the potential to protect the lysosomal membranes of cells during times of inflammatory activity. The extract was also shown to inhibit the denaturing of proteins caused by heat, which is a critical process associated with the damage of tissues caused by inflammation. The results of both experiments show that the extract has a concentration-dependent effect, with the IC_{50} values of the extract being lower than those of the standard anti-inflammatory drugs. The results of the above experiments can be attributed to the bioactive phytoconstituents of the extract, such as flavonoids and phenolic compounds. The results of the current study offer scientific evidence for the traditional use of Sida acuta in the treatment of inflammatory diseases and suggest that it may serve as a safe and natural alternative to NSAIDs. Further studies will be needed to validate the current findings.

Received: 20-03-2026
Accepted: 30-04-2026

Acta BIOCLÍNICA
ISSN Electrónico: 3105-403X

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Received: 20-03-2026
Accepted: 30-04-2026

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Thiyagarajan y Col.
Pg. 38-61

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Acta BIOCLÍNICA
ISSN Electrónico: 3105-403X

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Received: 20-03-2026
Accepted: 30-04-2026

Artículo Original

Volumen 16, N° 33. Julio-Diciembre 2026
Thiyagarajan y Col.
Pg. 38-61

DOI:

Acta BIOCLÍNICA
ISSN Electrónico: 3105-403X

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