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(RESEARCH ARTICLE)

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Evaluation of *in vitro* anti-inflammatory activity of aqueous leaf extract of *Capparis Brevis pina* Dc

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Abstract

Inflammation is an immune response triggered by pathogens, toxins, and cellular damage, leading to acute or chronic inflammatory conditions affecting multiple organs. The present study evaluates the in-vitro anti-inflammatory potential of the aqueous leaf extract of *Capparis brevis pina* DC (ALECB) using protein denaturation and membrane stabilization assays. The phytochemical screening of ALECB confirmed the presence of alkaloids, flavonoids, tannins, glycosides, and saponins, which are known for their pharmacological properties. The albumin denaturation assay demonstrated that ALECB exhibited dose-dependent inhibition of protein denaturation, with an IC_{50} of 93.877 µg/ml compared to 35.519 µ g/ml for Diclofenac. Similarly, in the HRBC membrane stabilization assay, ALECB showed an IC_{50} of 86.67 µg/ml, while Aspirin exhibited an IC_{50} of 37.81 µ g/ml. These findings suggest that ALECB possesses significant anti-inflammatory activity, although it is less potent than standard drugs. The observed effects can be attributed to the phytochemicals present in the extract, particularly flavonoids and phenolic compounds. The study highlights the potential of *Capparis brevis pina* DC as a natural anti-inflammatory agent, warranting further in-vivo investigations and bioactive compound isolation to explore its therapeutic applications.

Keywords: *Capparis brevis pina* DC; Inflammation; Anti-Inflammatory Activity; Albumin Denaturation; Membrane Stabilization; Phytochemicals

1. Introduction

1.1. What is Inflammation?

Inflammation is characterized as a localized response of living mammalian tissues to injury caused by various agents. This physiological reaction serves as a defense mechanism aimed at eliminating or containing harmful agents, followed by the removal of dead cells and tissues.[1] [Mohan, H. (2021)]

According to Lope et al. (1987), inflammation, also known as Phlogosis, is a response of the tissue blood vessels to aggressor agent that is characterized by the entry of liquids and cells into the interstitial space. According to Dassoler et al. (2004), the symptoms of an inflammatory reaction include discomfort, tumor(swelling), heat, Rubor (Redness), and loss of function. Although there are numerous reasons for inflammation, all of them share similar pathways. Phospholipase A2 is activated by the inflammatory agent's action on cell membranes, which releases arachidonic acid and its metabolites.[2]

1.2. Signs of inflammation

The roman writer Aulus Cornelius Celsus, in the 1st century A.D., identified the four cardinal signs of inflammation as:

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- Rubor - redness
- Tumour – swelling
- Calor – heat
- Dolor- pain

The fifth sign of inflammation, known as **functio laesa (loss of function)**, was later introduced by Virchow. The term, "inflammation" originates from the Latin word for burning, reflecting an ancient understanding, through burning is now recognized as just one of the signs of inflammation.[3]

1.3. Types of Inflammation

Inflammation can be classified into two main types based on the host's defense capacity and the duration of the response:

1.3.1. Acute inflammation

Acute inflammation is a short- term response lasting less than two weeks. It represents the body's immediate reaction to injury and typically resolves quickly, leading to healing. Key characteristics include:

- Accumulation of fluid and plasma at the affected site.
- Activation of platelets within blood vessels
- Presence of polymorph nuclear neutrophils as the primary inflammatory cells.

In some cases, this response can be severe and is referred to as fulminant acute inflammation.[4]

1.3.2. Chronic Inflammation

characterized by

- The presence of chronic inflammatory cells such as lymphocytes, plasma cells, and macrophages.
- Formation of granulation tissue.
- In certain cases, granulomatous inflammation.

Additionally, sub-acute inflammation refers to transitional phase between acute and chronic inflammation, typically lasting from 2 to 6 weeks.[5]

Table 1 Comparison between acute and chronic inflammation

	Acute Inflammation	**Chronic Inflammation**
Causative agent	Pathogens, injured tissues	Persistent acute inflammation due to non-degradable pathogens, persistent foreign bodies, or auto immune reaction.
Major cell	Neutrophils, mononuclear cells (monocytes, macrophages)	Mononuclear cells (monocytes, macrophages, lymphocytes, plasma cells), fibroblasts.
Primary mediators	Vasoactive amines, eicosanoids	IFN-γ and other cytokines, growth factors, reactive oxygen species, hydrolytic enzymes.
Onset	Immediate	Delayed
Duration	Few days	Up to many months, or years
Outcomes	Resolution, abscess formation, chronic inflammation	Tissue destruction, fibrosis

1.3.3. Introduction about Capparis brevis pina DC

- **Botanical information:** *Capparis brevis pina* DC, a significant medicinal plant in India, belongs to the Capparis genus, which is a key part of the Capparaceae family. [6]

This plant is characterized as a shrub, notable for its thorny stipules, tough, leathery leaves, and distinct white flowers. The berries of *Capparis brevis pina DC* have an ovoid/ellipsoid shape.[7] *Capparis brevis pina* DC is one of the examples for secondarily balanced tetraploids.[8]

The Capparaceae family, which includes this particular plant, is primarily located in tropical areas. This species is indigenous to the region that stretches from India to Assam and Sri Lanka, where it typically grows as a shrub or tree, especially in the seasonally dry tropical biome.[9] This family features a variety of plant forms, such as herbs, shrubs, and occasionally small trees. Many of these species, whether they grow upright or climb, are well-suited to warm environments. Globally, the Capparaceae family is notably diverse, consisting of around 39 genera and roughly 650 species. This extensive distribution highlights the ecological and botanical importance of the family, particularly in warmer regions of the world.[10] The significance of *Capparis brevis pina* DC as a medicinal plant in India emphasizes the crucial role that Capparaceae plants play in traditional medicine, especially in areas rich in biodiversity and established herbal practices. This species, with its distinctive physical traits and therapeutic benefits, adds to the rich and valuable botanical legacy of the Capparaceae family.

1.3.4. Taxonomy

- Root: Root
- Kingdom: Plantae
- Phylum: Tracheophyta
- Class: Magnoliopsida
- Order: Capparales
- Family: Capparaceae
- Genus: Capparis
- Species: *Capparis brevis pina* DC. [11]

The Indian caper scientifically known as *Capparis Brevis pina* DC has different vernacular names in different parts of India, indications its wide acceptance and use in traditional practices.

In Tamil it is called as Adanda, kattukanji. In Oriya, they are called by names such as Lepura, Lephra, Nepheda and Nipura. In Malayalam it is known as Chedimukanthi and In Marathi, the Indian caper is referred to as vagathi.[12]

- **Chemical constituents:** The chemical components identified in the *Capparis brevis pina* DC species include alkaloids, flavonoids, saponins, and terpenoids. Research has indicated that this species exhibits antioxidant activity, as demonstrated through assays such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and phosphomolybdenum.[13]

Figure 1 Leaf of *Capparis brevis pina* DC

Figure 2 Plant of *Capparis brevis pina* DC

- **Pharmacological properties of Capparis species**: Species such as *Capparis mooni* and *Capparis tomentosa* are reported to treat tuberculosis.[14] *Capparis decidua* is used to treat diabetes and hypercholesterolemia.[15] The seed of *Capparis separia* and fruit of *Capparis zeylanica* were used as antidotes for snake bite.[16]

Recently more interest has been raised towards *Capparis spinosa* due to its large amount of bio active ingredients.it is found to have various pharmacological activities like antioxidant, anti-microbial, rheumatism, gout, anti-cancer and hepatoprotective effects. *Capparis spinosa* possesses anti-tubercular properties and is widely used in ayurvedic preparations to treat acute viral hepatitis and cirrhosis.[17]

1.4. Advantages of using *Capparis Brevispina* DC

- Antioxidant activity: studies have shown that leaf extracts of *Capparis brevispina* DC exhibit significant anti-oxidant properties which helps in combating oxidative stress and reducing cell damage.
- Cytotoxic effects: the plant has demonstrated by cytotoxic activities, suggesting a potential role in fighting cancer cells.
- Phytochemical Richness: *Capparis brevispina* DC is a source of valuable phytochemical compounds that can have various physiological functions, contributing to overall health and well- being. [18]
- Medicinal Potential: The plant's extracts have shown antimicrobial properties, indicating possible applications in fighting against harmful microorganisms. These advantages highlight the potential of *Capparis brevispina* DC as a natural remedy with diverse health benefits.[19]

2. Methodology

2.1. Preparation of plant extract

- A 40g sample of *Capparis brevispina* DC leaf powder was enclosed in a porous bag made from clean cloth of strong filter paper and securely fastened.
- The extraction solvent, specifically water was poured into the round bottom flask, followed by placing the thimble into the extraction chamber.
- In another beaker, the solvent was heated to generate vapor which was condensed and returned as liquid into the extraction chamber.
- Once the solvent in the extraction chamber reached the siphon level, the solvent along with the extract returned to the flask.
- When the solvent rose to the siphon level in the extraction chamber, it flowed back into the flask along with the extract.
- This cycle continued until it was clear that the compound had been completely extracted, as indicated by the absence of any residue flowing with the solvent from the extraction chamber.[20]

The percentage yield of the plant extract from the plant material can be calculated using the formula:

Percentage yield = (Weight of extract obtained) / Weight of plant material used) X 100

2.2. Qualitative phytochemical investigation:[21]

Qualitative phytochemical tests were conducted for the test extracts to list out and identify the various phytoconstituents.

- Test for Steroids and Triterpenoids: Liebermann–Burchard Test
- Test for Glycosides: Borntrager's Test and Bromine Water Test
- Test for Saponins: Foam Test
- Test for Alkaloids: Hager's Test , Dragendorff's Test , Mayer's Test and Wagner's Test
- Test for Flavonoids: Ferric Chloride Test , Alkaline Reagent Test and Lead Acetate Test
- Test for Tannins: $FeCl_3$ Test
- Test for Proteins: Biuret Test

2.3. Assessment of *in vitro* anti-inflammatory activity

2.3.1. Method 1: Inhibition of albumin denaturation

The anti-inflammatory activity of *Capparis brevis pina* DC was studied by using inhibition of albumin denaturation technique which was studied. The reaction mixture was consists of test extracts and 5% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of 1N HCl. The sample extracts were incubated at 37ºC for 20 min and then heated to 51º C for 30 min and the temperature was measured and maintained using a thermometer. After cooling the samples the turbidity was measured at 660nm of wavelength. (UV Visible Spectrophotometer Shimadzu) The experiment was performed in triplicate.[22]

The Percentage inhibition of protein denaturation was calculated as follows:

$$\text{Percentage Inhibition (\%)} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs Control}} \text{x } 100$$

2.3.2. Method 2 : Heat induced hemolysis

The reaction mixture consisted of test sample of different concentrations (10 - 50 µg/ml) and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Aspirin was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56 ºC for 30min and the temperature was measured and maintained using a thermometer. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm of wavelength. The experiment was performed in triplicates for all the test samples.[23,24]

The Percentage inhibition of Hemolysis was calculated as follows:

$$\text{Percentage Inhibition (\%)} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs Control}} \text{x } 100$$

3. Result

The percentage yield of the plant extract from the plant material was determined using the formula:

Percentage yield = (Weight of extract obtained × 100) / (Weight of plant material used).

In this study, 40 grams of plant material were used, and 3 grams of extract were obtained. Substituting these values into the formula:

Percentage yield = (3 × 100) / 40 = 7.5%.

Thus, the percentage yield of the plant extract was calculated to be 7.5%. This yield provides insight into the efficiency of the extraction process and the potential availability of bioactive compounds from the plant material.

3.1. Preliminary phytochemical screening

Table 2 Phytochemical tests for ALECB

S.no	Phytochemicals	Test	Results Positive/Negative
1.	Alkaloids	Mayer's test	+
		Wagner's test	+
		Dragendroff's test	-
		Hager's test	+
2.	Flavonoid	Alkaline reagent test	+
		Lead acetate test	+
3.	Glycosides	Borntrager's test	-
		Bromine water test	+
4.	Proteins	Millon's test	+
		Ninhydrin test	-
5.	Phenols/tannins	FeCl test	+
		Lead acetate test	+
6.	saponins	Foam test	+

(+) Indicates positive result, (-) indicates negative result.

3.2. Inhibition of albumin denaturation

Protein denaturation refers to the alteration of proteins' tertiary and secondary structures due to external factors or agents, including strong acids or bases, concentrated inorganic salts, organic solvents, or heat. Typically, when proteins undergo denaturation, they lose their biological functionality. This process is recognized as a significant contributor to inflammation. In exploring the mechanisms behind anti-inflammatory activity, research was conducted on the capacity of plant extracts to prevent protein denaturation, demonstrating effectiveness in inhibiting heat-induced denaturation of albumin.

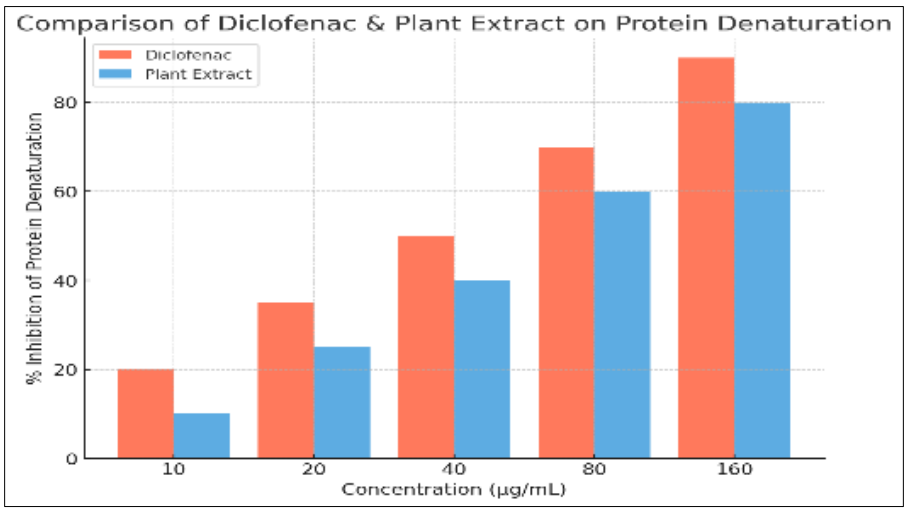

Figure 3 % inhibition of Diclofenac and ALECB on Protein denature

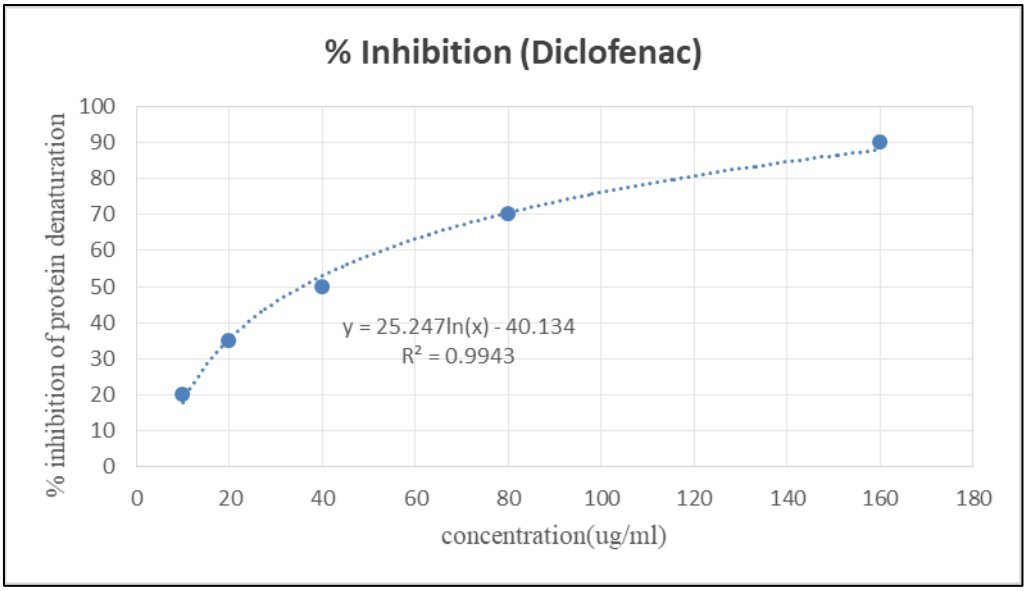

Figure 4 IC_{50} Graph for Diclofenac (Protein Denaturation Assay)

IC_{50} =35.519

"The dose-response curve of Diclofenac in the albumin denaturation assay shows an IC_{50} value of 35.519 µg/ml. The inhibition percentage increases with concentration, indicating its potent anti-inflammatory activity."

IC_{50} Graph for ALECB (Protein Denaturation Assay)

IC 50 =93.877

"The inhibition curve for ALECB in the albumin denaturation assay demonstrates a dose-dependent inhibition of protein denaturation, with an IC_{50} of 93.877 µg/ml, suggesting moderate anti-inflammatory potential."

3.3. Heat Induced Hemolysis

The stabilization of the HRBC membrane has been employed as a technique to investigate in vitro anti-inflammatory activity, given that the erythrocyte membrane shares similarities with the lysosomal membrane. This stabilization suggests that the extract may also be effective in stabilizing lysosomal membranes. Such stabilization is crucial for mitigating the inflammatory response, as it prevents the release of lysosomal components from activated neutrophils, including bacterial enzymes and proteases, which can exacerbate tissue inflammation and damage when released into the extracellular environment. The enzymes released from lysosomes during inflammation are associated with various disorders, and their extracellular activity is linked to both acute and chronic inflammatory conditions. Non-steroidal anti-inflammatory drugs function by either inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. The extract was effective in inhibiting the heat induced hemolysis at different concentrations.

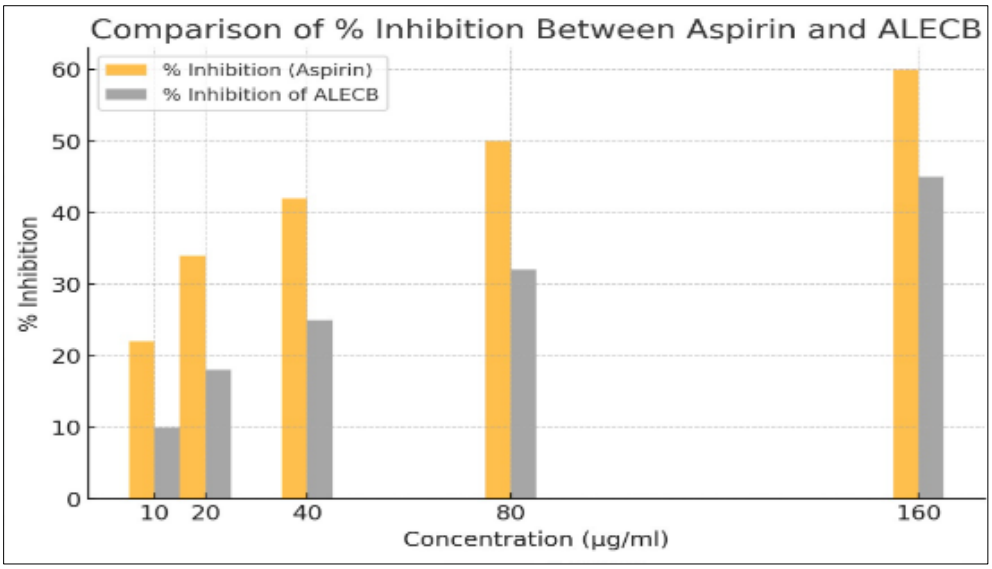

Figure 5 % inhibition of Aspirin and ALECB on Heat induced haemolysis

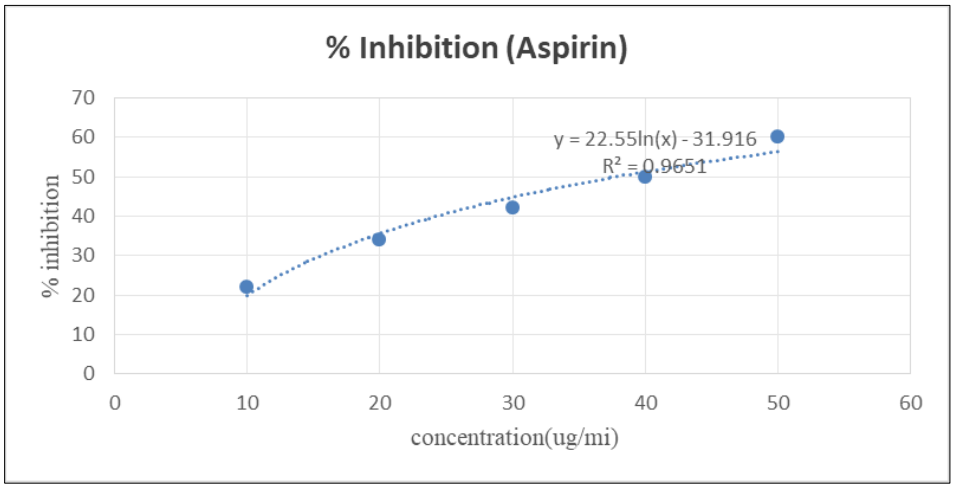

Figure 6 IC_{50} Graph for Aspirin (HRBC Membrane Stabilization Assay)

IC_{50} = 37.81

"Aspirin exhibits a concentration-dependent inhibition of heat-induced hemolysis in the HRBC membrane stabilization assay, with an IC_{50} of 37.81 µg/ml, indicating its strong anti-inflammatory activity."

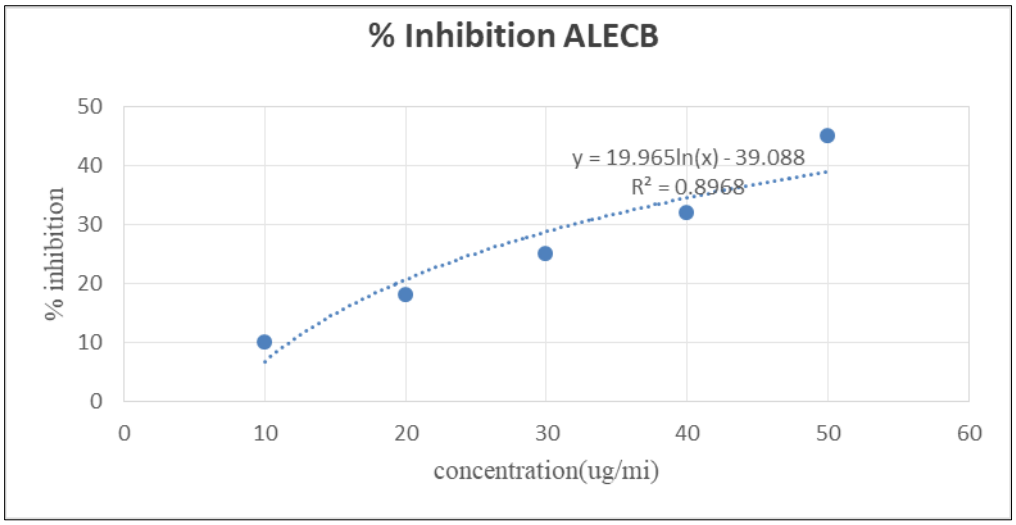

Figure 7 IC_{50} Graph for ALECB (HRBC Membrane Stabilization Assay)

IC_{50} = 86.67

"The HRBC membrane stabilization assay for ALECB shows an IC_{50} value of 86.67 µg/ml, demonstrating its ability to stabilize cell membranes and reduce hemolysis, albeit with lower potency compared to Aspirin."

4. Discussion

4.1. Preliminary Phytochemical Screening

The preliminary phytochemical screening of ALECB revealed multiple bioactive compounds. Alkaloids were confirmed by Mayer's, Wagner's, and Hager's tests but not by Dragendorff's test, indicating potential pharmacological properties. Flavonoids tested positive in the alkaline reagent and lead acetate tests, suggesting strong antioxidant and anti-inflammatory effects. Glycosides showed mixed results, being positive in the bromine water test but negative in Borntrager's test, which may influence cardiac health. Proteins were detected by Millon's test but not by Ninhydrin, indicating enzymatic roles. Phenols/Tannins tested positive in $FeCl_3$ and lead acetate tests, confirming polyphenolic antioxidant properties. Saponins were identified using the foam test, suggesting surfactant properties that may stabilize cell membranes. These bioactive compounds contribute to ALECB's potential therapeutic effects. The presence of these compounds indicates possible antimicrobial, anti-inflammatory, and antioxidant activities.

4.2. Inhibition of Albumin Denaturation (Anti-inflammatory Activity)

Protein denaturation is a key factor in inflammation. The ability of a substance to prevent heat-induced denaturation of albumin indicates its potential anti-inflammatory effects.

- The standard drug Diclofenac showed strong inhibition of protein denaturation, reaching 90% inhibition at 160 µg/ml with an IC_{50} of 35.519 µg/ml.
- ALECB also exhibited a dose-dependent inhibition of protein denaturation, with 80% inhibition at 160 µg/ml and an IC_{50} of 93.877 µg/ml.
- The higher IC_{50} of ALECB compared to Diclofenac suggests that while the extract possesses anti-inflammatory activity, it is less potent than Diclofenac.
- These results indicate that ALECB has notable anti-inflammatory potential, likely due to its phytochemical constituents, particularly flavonoids and phenols, which are known to stabilize proteins and reduce denaturation.

4.3. Heat-Induced Hemolysis (Membrane Stabilization)

The HRBC (Human Red Blood Cell) membrane stabilization assay is an important indicator of anti-inflammatory potential, as it simulates the lysosomal membrane stabilization mechanism.

- Aspirin, a known anti-inflammatory drug, showed 60% inhibition at 50 µg/ml with an IC_{50} of 37.81 µg/ml.

- ALECB exhibited a concentration-dependent effect, reaching 45% inhibition at 50 µg/ml, with an IC_{50} of 86.67 µg/ml.
- While the effect of ALECB was lower than that of Aspirin, it still exhibited significant activity. The presence of flavonoids and tannins in the extract may contribute to its membrane stabilization properties by preventing oxidative damage and inhibiting the release of inflammatory mediators.

Table 3 Comparative analysis of anti-inflammatory effects of parameter Diclofenac (standard) and ALECB (Aqueous leaf extract of *Capparis brevis pina* DC)

Parameter	Diclofenac	ALECB
% inhibition of Albumin denaturation(160 µg/ml)	90%	80%
IC_{50} (Albumin denaturation)	35.519 µg/ml	93.877 µg/ml
% inhibition of HRBC haemolysis (50 µg/ml)	60%	45%
IC_{50}(HRBC membrane stabilization)	37.81 µg/ml	86.67 µg/ml

5. Conclusion

The phytochemical analysis confirmed the presence of alkaloids, flavonoids, tannins, glycosides, and saponins, which may contribute to the anti-inflammatory properties of ALECB. ALECB demonstrated significant anti-inflammatory activity compared to standard drug.

The albumin denaturation assay and hemolysis assay both showed dose-dependent inhibition, suggesting the extract's potential in reducing inflammation. Although ALECB was less potent than standard drugs, its natural origin and bioactive composition highlight its potential for further research in herbal anti-inflammatory treatments.

Future studies should include in vivo validation, bioactive compound isolation, and mechanistic studies to fully establish the therapeutic potential of ALECB in inflammatory conditions.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no known financial, personal, or professional conflicts of interest that could have influenced the work reported in this manuscript.

Additionally, the authors confirm that they have no affiliations with or involvement in any organization or entity with a direct financial interest in the subject matter or materials discussed in this manuscript.

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