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# Assessment of Anti- Inflammatory Potential of *Caesalpinia Crista* Seeds by In-Vitro Bovine Serum Albumin Denaturation Method

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**ABSTRACT**

Inflammation represents a protective biological response to tissue injury and is implicated in various chronic disorders, including arthritis. Protein denaturation constitutes a primary cause of inflammation by generating auto-antigens and inflammatory mediators. This study aimed to evaluate the *in-vitro* anti-inflammatory activity of *Caesalpinia crista* seed extract using the bovine serum albumin (BSA) protein denaturation assay. The ethanolic extract of Caesalpinia crista seeds was prepared and tested at different concentrations. The reaction mixture consisted of bovine serum albumin as the protein source, phosphate buffered saline to maintain pH, and varying concentrations of the plant extract. Indomethacin was used as the standard reference drug. Denaturation of protein was induced by heat and the absorbance was measured spectrophotometrically to calculate the percentage inhibition of protein denaturation. The extract showed a concentration-dependent inhibition of protein denaturation, with inhibition of increasing values. The standard drug Indomethacin exhibited slightly higher inhibition at the same concentrations. The activity of the extract was found to be comparable to the standard drug at higher concentrations.

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## 1. INTRODUCTION

Inflammation serves as the body's primary defense mechanism, involving immune cells, blood vessels, and molecular mediators. It is triggered when body cells encounter stress from physical agents, chemicals, or microbes, manifesting as redness, swelling, heat, pain, and loss of function in the affected area. Damaged cells release mediators such as prostaglandins, kinins, and histamines, which promote vasodilation and increased capillary permeability. This process also enhances protein denaturation and induces alterations in cell membranes.(1)

Denaturation is a biochemical process in which a protein loses its native three-dimensional structure, specifically through alterations in its secondary, tertiary, or quaternary structures. This disrupts non-covalent interactions like hydrogen bonds, ionic bonds, hydrophobic interactions, and disulfide bridges—though the term in your query mentions covalent bonds, denaturation primarily affects weaker non-covalent bonds while leaving the primary structure (peptide bonds) intact. The protein unfolds into a random coil, losing its biological function without breaking the amino acid sequence.(2)

Changes in a protein's structure are generally accompanied by alterations in its chemical, physical, and functional properties. Numerous studies have shown that many proteins or enzymes lose their activity either irreversibly or reversibly, when subjected to various natural or man-made conditions. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and lactate dehydrogenase (LDH) are examples of enzymes/proteins that lose their properties when subjected to low temperatures.

Protein denaturation refers to an intramolecular change in a protein's structure, which can be studied by examining the displacement and relocation of its constituent groups and atoms.(2)

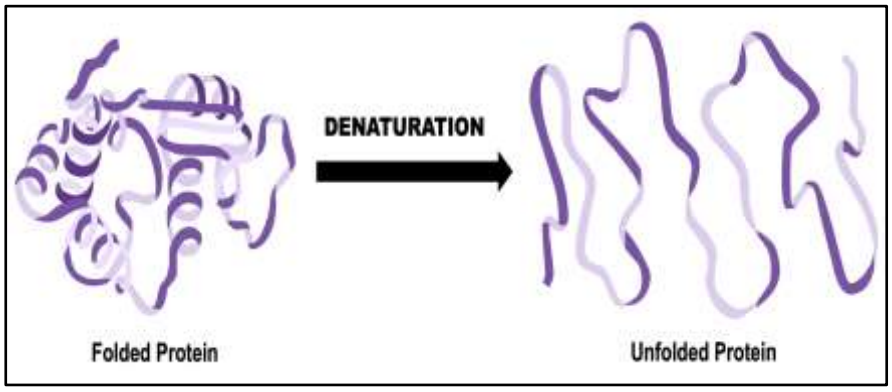


**Fig.1: Protein denaturation[3]**

## 2. EXPERIMENTAL PROTOCOL

2.1 Materials And Methods

*Caesalpinia crista* is a medicinal herb of the family Caesalpiniaceae found in subtropical and tropical regions of Southeast Asia. Commonly known asFever nut.It is a renowned medicinal plant in Ayurveda, one of the varieties of the Karanja commonly used for its diverse therapeutic properties.[4]

Recent research has confirmed the therapeutic potential of this plant, as potent anti-inflammatory, antipyretic and antimicrobial properties. Various studies have shown that it exhibits significant antioxidant activities, which contribute to its use in treating conditions like diabetes and inflammatory disorders.[5]

### 2.1.1. Collection of Plant Materials and Extraction[6]

**Source:** Seeds of *Caesalpinia crista.*

1. **Seed collection:** Harvest fresh, mature seeds from healthy *Caesalpinia crista* plants. Select intact seeds free from damage, mold, or infestation.
2. **Washing:** Rinse seeds thoroughly under running distilled water to remove dirt, debris, or impurities. Agitate gently for 5-10 minutes, then drain on sterile filter paper.
3. **Drying:** Air-dry seeds in shade at room temperature (25-30°C) for 7-10 days, or until completely dry (moisture content <10%, verified by weight stability).
4. Spread in a single layer on clean trays in a well-ventilated area.
5. **Grinding:** Grind dried seeds into a fine powder (particle size 40-60 mesh) using a mechanical grinder or mill.
6. Avoid overheating by processing in small batches.
7. **Storage:** Transfer powder to airtight, opaque containers. Store in a cool (4-25°C), dark, dry place.
8. Label with date, batch details, and expiration (typically 6-12 months).

### 2.1.2. Solvent Extraction[6]

This protocol describes a sequential extraction method using solvents of increasing polarity to separate compounds based on their solubility.

Materials:

- Powdered seed material of *Caesalpinia crista*
- Petroleum ether
- Ethanol
- Soxhlet apparatus
- Filter paper
- Glasswares (beakers, flasks etc..)

Procedure:

Dried seed kernels of *Caesalpinia crista* were used. The seed coat was manually broken, and the testa was separated. The kernels were powdered and passed through sieve No. 40. The powder was stored in an airtight container. Place the powdered material in a thimble and insert it into the main chamber of the soxhlet apparatus. Pour the ethanol in a round bottom flask (RBF). Heat the solvent to its boiling point and carry out the extraction. The powder was defatted with petroleum ether in a Soxhlet apparatus for 24 hours. After defatting, the powder was dried and recharged into the same Soxhlet extractor. It was then extracted with ethanol for 16 hours. The ethanolic extract yielded a reddish-brown sticky mass.[7]

Chemicals:

- Bovine Serum Albumin(BSA)
- Phosphate buffered saline
- Standard drug-Indomethacin
- Extract (powdered *Caesalpinia crista*)

### 2.2. METHOD

**Test solution:** it consists of 0.45ml of bovine serum albumin and 0.05ml of test solution of various concentrations.

**Test control solution:** it consists of 0.45ml of bovine serum albumin and 0.05ml of dimethyl sulfoxide.

**Standard solution:** it consists of 0.45ml of bovine serum albumin and 0.05ml of indomethacin of various concentrations.

### 1.6 Procedure[1]

- One percent aqueous solution of bovine serum albumin was prepared.
- 0.05ml of various concentrations (100, 200, 400, 800,1000) of test drugs and standard drug

indomethacin was taken respectively and 0.45ml of bovine serum albumin solution mixed. The samples were incubated 20 minutes at 37ºC and heated for 5 minutes at 51ºC.

- 2.5ml of phosphate buffer was added, after the samples were cooled.
- The absorbance was measured at 660nm by using a UV spectrophotometer.
- The protein denaturation inhibition percentage was determined by the following formula:

$$\%\ \text{inhibition} = \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$$

## 3.RESULTS AND DISCUSSION

Preliminary phytochemical screening of the ethanolic seed extract of *Caesalpinia crista* was carried out to find the various phytoconstituents. This preliminary screening indicated the presence of major secondary metabolites including carbohydrate, flavonoids, alkaloids, tannins, saponins, coumarin glycosides, reducing sugars, triterpenoids and fatty acids and proteins.

The protein denaturation assay using bovine serum albumin (BSA) was used to assess the *in-vitro* anti-inflammatory activity of the ethanolic extract, with results compared to the standard drug indomethacin. BSA was chosen due to its high sensitivity to heat-induced denaturation and its similarity to human physiological proteins.

**Table 1: Percentage inhibition of Bovine Serum Albumin Denaturation by seed extract of *Caesalpinia crista*.**

| | CONCENTRATION(µg/ml) | ABSORBANCE(nm) | TEST CONTROL | % INHIBITION |
|---|---|---|---|---|
| **TEST (Ethanolic extract)** | 100 | 0.063 | 0.059 | 6.77 |
| | 200 | 0.069 | | 16.90 |
| | 400 | 0.077 | | 30.50 |
| | 800 | 0.086 | | 45.70 |
| | 1000 | 0.092 | | 55.90 |
| **STANDARD (Indomethacin)** | 100 | 0.101 | 0.094 | 7.40 |
| | 200 | 0.114 | | 21.27 |
| | 400 | 0.127 | | 35.10 |
| | 800 | 0.138 | | 46.80 |
| | 1000 | 0.148 | | 57.44 |

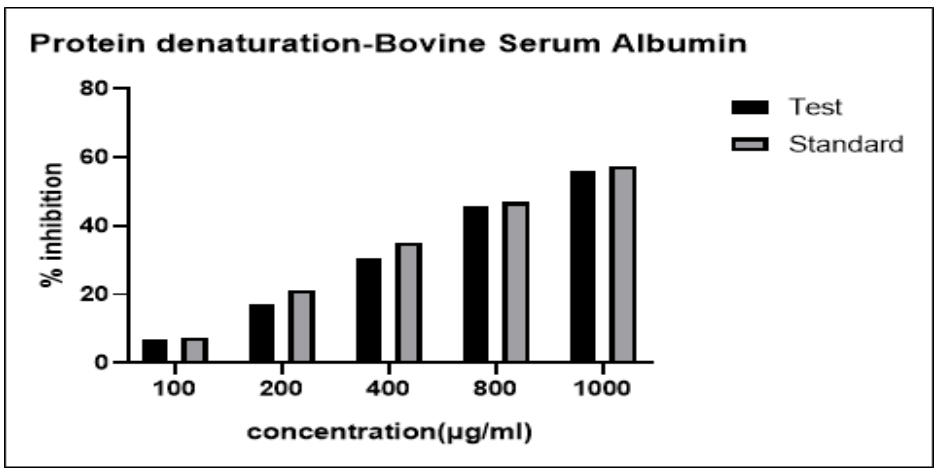


**Figure 1: Effect of seed extract and indomethacin on Bovine Serum Albumin Denaturation.**

The ethanolic extract demonstrated concentration-dependent inhibition of protein denaturation, with minimal activity at 100 µg/mL and marked inhibition at 800–1000 µg/mL, reaching a maximum of 55.9%. Indomethacin exhibited comparable activity, achieving maximum inhibition of 57.44%. Overall, the extract's performance was similar to the standard, indicating promising anti-inflammatory potential.

Here, the test solution exhibited an $IC_{50}$ of 880 µg/mL compared to 890 µg/mL for the standard, indicating comparable anti-inflammatory activity with the test sample slightly outperforming the standard drug.

The graph depicts the *in-vitro* anti-inflammatory activity of the test extract and standard drug (indomethacin) in the bovine serum albumin protein denaturation assay. Both exhibited concentration-dependent increases in percentage inhibition from 100 to 1000 µg/mL.

The test extract exhibited inhibition increasing from approximately 7% to 56%, while the standard (indomethacin) showed slightly higher inhibition, from 8% to 58%. At higher concentrations, the extract's activity was nearly comparable to indomethacin, indicating significant anti-inflammatory potential.

These results indicate that the extract effectively inhibits protein denaturation, likely due to phytochemicals such as flavonoids and phenolics, supporting its potential as an anti-inflammatory agent.

## 4. CONCLUSION

The present study demonstrated the *in-vitro* anti-inflammatory activity of *Caesalpinia crista* seed extract using the bovine serum albumin protein denaturation assay, exhibiting concentration-dependent inhibition of protein denaturation with maximum inhibition at the highest concentration tested. The extract's ability to prevent heat-induced protein denaturation suggests its potential to stabilize proteins and mitigate inflammatory responses. This activity may be attributed to phytoconstituents such as flavonoids, phenolics, and tannins, which are known for their anti-inflammatory and antioxidant properties. These findings indicate that *Caesalpinia crista* possesses significant anti-inflammatory potential and may serve as a promising natural source for developing safer anti-inflammatory agents.

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