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Review

Lipoxygenase inhibition potential of *Caesalpinia crista:* An *in vitro* study.

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Abstract

Lipoxygenases are oxidative enzymes involved in the metabolism of polyunsaturated fatty acids like linoleic acid and arachidonic acid that result in the formation of lipid hydroperoxides. In uncontrolled lipoxygenase enzyme activity leads to the generation of inflammatory mediators and also oxidative stress. In humans these enzymes have a major role in the biosynthesis of pro-inflammatory compounds that are involved in the development of chronic inflammatory conditions and also diseases such as cardiovascular diseases such as stroke and heart attack and neurodegenerative disorders. So inhibiting lipoxygenase activity has become a key approach for managing inflammatory disorders.

So based on this, an investigation was conducted to examine the in vitro inhibitory test potential of the test substance (*Caesalpinia crista*) using a spectrophotometric enzyme inhibition assay. And the test substance exhibited potent inhibition of the lipoxygenase enzyme when compared with the standard drug (Quercetin).

Keywords: Lipoxygenases, linoleic acid, arachidonic acid, lipid hydroperoxides, *Caesalpinia crista,* Quercetin.

1. INTRODUCTION

Ceasalpinia crista, a medicinal shrub of the *Ceasalpiniaceae* family, is widely distributed throughout the tropical and subtropical regions of Southeast Asia. Known as Latakaranja in Ayurveda, it is a prominent variety of karanja traditionally utilized for its broad spectrum of therapeutic properties.

Recent research has confirmed the therapeutic potential of this plant as a potent anti-inflammatory, antipyretic, and antimicrobial. Various studies have shown that it exhibits significant antioxidant activities, which

contribute to its use in treating conditions like diabetes and inflammatory disorders.

C. crista is used in treating a vast range of diseases. Different parts of the plant, like leaves, flowers, fruit, root, bark, seeds, and seed oil, were used medicinally. Roots were used in the treatment of tumors, smallpox, colic fever, malaria, menstrual complaints, pulmonary tuberculosis, uterine disorders, diabetes, and asthma. They were used as diuretics. The seeds were considered as fugal, periodic, tonic, and vesicant. They are used to treat colic, convulsions, leprosy, and palsy. The oil from the seeds is said to soften the skin and remove pimples.

Here lipoxygenases (LOX) are critical oxidative enzymes that stimulate the release of cytokines and mediate inflammation. Inhibition of these enzymes is a crucial approach to preventing the progression of inflammatory illnesses. While the leaves of *Ceasalpinia crista* have shown promising 5-lipoxygenase inhibition, studies focusing specifically on the seed extract's ability to inhibit LOX are limited. Therefore, this article presents the lipoxygenase inhibition assay of *Ceasalpinia crista* seed to establish its potential as a natural, safe, and effective therapeutic agent.

2.EXPERIMENTAL PROTOCOL

2.1. MATERIALS AND METHODS

Ceasalpinia crista, a medicinal shrub of the *Ceasalpiniaceae* family, is widely distributed throughout the tropical and subtropical regions of Southeast Asia. Known as Latakaranja in Ayurveda, it is a prominent variety of karanja traditionally utilized for its broad spectrum of therapeutic properties.

2.1.1COLLECTION OF PLANT MATERIALS AND EXTRACTION

SOURCE: SEEDS OF *CEASALPINIA CRISTA*

1. Seed procurement: Seeds of *Caesalpinia crista* were procured from a local medicinal plant supplier. Healthy, mature seeds free from physical damage, fungal growth, and infestation were selected.

2. Washing: The seeds were rinsed thoroughly under running distilled water to remove adhering dust, dirt, and impurities. They were gently agitated for 5-10 minutes and then drained on sterile filter paper.

3. Drying: The washed seeds were shade-dried at room temperature (25-30 degrees Celsius) for 7 to 10 days until completely dry, confirmed by constant weight.

4. The seeds were spread in a single layer on clean trays and dried in a well-ventilated area.

5. Grinding: The dried seeds were ground into a fine powder using a mechanical grinder.

6. Storage: The powdered material was transferred into airtight, opaque containers and stored in a cool, dark, and dry place.

2.1.2 SOLVENT EXTRACTION

The powdered material was placed in a Soxhlet extraction apparatus. This apparatus was assembled using a round-bottom flask, Soxhlet extractor, and condenser supported by stands and clamps. Also, this extraction was conducted using ethanol as a solvent to separate compounds based on their solubility.

Materials

- Powdered seed material of *Ceasalpinia crista*
- Ethanol
- Petroleum ether
- Filter paper
- Glassware (flask, beaker, etc.)
- Soxhlet apparatus

Procedure:

As mentioned earlier in this Soxhlet extraction, we used ethanol as a solvent and placed it in the round-bottom flask, which was heated using an isomantle. Also, the powder was defatted with petroleum ether in a Soxhlet apparatus for 24 hours.

The powdered seed material was packed into a cellulose thimble and placed inside the extractor. On heating, ethanol evaporated, condensed in the condenser, and percolated through the plant material. When the solvent reached the siphon level, the extract was siphoned back into the round-bottom flask, completing one extraction cycle. After completion of extraction, the apparatus was cooled, and the ethanolic extract was collected (reddish-brown sticky mass) and stored in a labeled container.

CHEMICALS USED IN LIPOXYGENASE INHIBITION METHOD

- Soybean lipoxygenase enzyme
- Linoleic acid
- Borate buffer (PH 9)
- Quercetin (standard drug)
- Extract (powdered *Caesalpinia crista)*

3. METHOD

SPECTROPHOTOMETRIC ASSAY FOR 5-LOX INHIBITION.

Principle: This assay is based on the enzymatic activity of 5-LOX on a polyunsaturated fatty acid substrate like linoleic acid. This enzyme converts linoleic acid to a hydroperoxy derivative compound; this compound strongly absorbs light at a wavelength of 660 nm. If there is a presence of an inhibitor, the absorbance gets decreased.

Assay procedure:

1. TEST SOLUTION: It consists of 0.25 ml of enzyme solution and 1 ml of various test solutions of different concentrations varying from 20 mg/ml to 100 mg/ml, and 1 ml of linoleic acid and borate buffer.
2. CONTROL SOLUTION: It consists of 0.25 ml of enzyme solution and 1 ml of linoleic acid and borate buffer.
3. STANDARD SOLUTION: Contains a known concentration of quercetin and enzyme solution, buffer solution, and linoleic acid.

Working procedure

- A stock solution of 5 lipoxygenases was prepared at a concentration of 5 mg/ml.
- Then 1 ml of various test solutions of different concentrations, varying from 20 mg/ml to 100 mg/ml, was dissolved in 0.25 ml of enzyme solution.
- 1 ml of linoleic acid was added and incubated for 5 minutes at 25 degrees Celsius.
- Then 2.5 ml of borate buffer was added
- The absorbance was measured at 660 nm by using a UV spectrophotometer.
- Calculation of inhibition

$$\%\text{LOX inhibition} = (A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}$$

Also, a control reaction was performed under identical conditions without the test compound

4. RESULTS

IC50 VALUE

The calculated IC50 value for the lipoxygenase inhibition assay of the **test** sample was found to be **70 µg.**

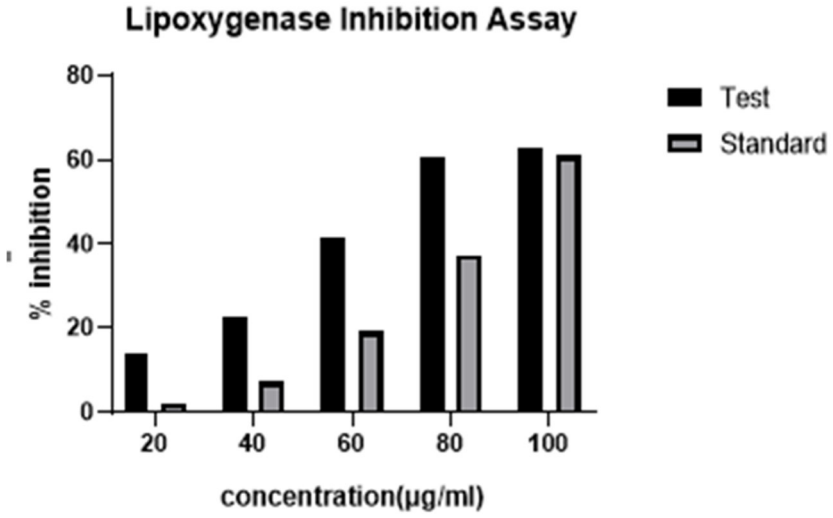
The calculated IC50 value for the lipoxygenase inhibition assay of the standard was found to be **91 µg.**

These results indicated that the test sample exhibits stronger lipoxygenase inhibitory activity than the standard, as reflected by its lower IC50 value (70 µg). A lower IC50 value suggests that a smaller concentration of the test sample is required to achieve 50% inhibition compared to the standard (91 µg).

LIPOXYGENASE INHIBITION ASSAY

TEST (Ethanolic extract)	CONCENTRATION	ABSORBANCE	TEST CONTROL	% INHIBITION
	20	0.544	0.632	13.92
	40	0.488		22.78
	60	0.368		41.77
	80	0.249		60.60
	100	0.233		63.13

STANDARD (Quercetin)	20	0.384	0.392	2.04
	40	0.364		7.14
	60	0.317		19.13
	80	0.245		37.50
	100	0.153		60.96



5. CONCLUSION

- The lipoxygenase inhibition assay showed that the ethanolic extract exhibited a concentration-dependent inhibitory activity against the lipoxygenase enzyme.
- As the concentration increased from 20 to 100 µg, the percentage inhibition significantly increased, indicating good anti-inflammatory potential.
- When compared with the standard quercetin, the test extract showed comparable and appreciable inhibition at higher concentrations.
- These results suggest that the extract contains bioactive compounds capable of inhibiting lipoxygenase and may serve as a potential anti-inflammatory agent.

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