Evaluation of Lipoxygenase Inhibitory Potential of the Ethanol Fraction from Star Fruit (Averrhoa carambola L.) Leaves Sourced from Assam.

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ABSTRACT:

Averrhoa carambola L. leaves have long been utilized in traditional medicine. This study aimed to assess the anti-inflammatory potential of *A. carambola* L. leaf extracts through a lipoxygenase inhibition assay, focusing on the ethanol fraction derived from leaves collected in Ahomgaon, Assam. The extraction process was carried out using the Soxhlet method, initially with petroleum ether, followed by 95% ethanol. The ethanol fraction was then evaluated for its lipoxygenase inhibitory activity using linoleic acid as the substrate, while its total flavonoid and total phenol contents were also quantified. The correlation between IC_{50} values and the total flavonoid and total phenol contents in the ethanol fraction was analyzed. Results showed that the ethanol fraction inhibited lipoxygenase enzyme activity, with an IC_{50} value of 96.86 µg/mL. The total flavonoid content in the ethanol fraction was determined to be 1.52 mg of quercetin equivalents per gram of extract, whereas the total phenol content measured 8.323 mg of gallic acid equivalents per gram of extract. A strong negative correlation was observed between IC_{50} values and the total flavonoid and total phenol contents, with correlation coefficients of −0.701 and −0.603, respectively, suggesting that higher flavonoid and phenol contents corresponded to lower IC50 values.

***Keywords*:** *Averrhoa carambola* L., Inflammation, Lipoxygenase, Flavonoid, Phenol.

INTRODUCTION:

Star fruit (*Averrhoa carambola* L.) leaves have been traditionally used to treat inflammatory skin conditions such as eczema, boils, and pyoderma, as well as skin infections caused by bacteria, viruses (smallpox), and fungi (ringworm) [1-3]. Lipoxygenase is an enzyme responsible for the biosynthesis of inflammatory mediators like leukotrienes, which contribute significantly to the development of inflammatory diseases such as asthma [4-6]. Natural compounds, particularly flavonoids such as flavones found in *A. carambola*, have shown potential anti-inflammatory properties [7]. Previous research has reported an IC_{50} value of 2.08 ppm for apigenin against lipoxygenase activity and an IC_{50} value of 7.84 ppm for the ethyl acetate fraction of star fruit, suggesting its potential for preventing skin edema and its use in cosmetic formulations as a skin-soothing agent [8]. The objective of this study was to evaluate the lipoxygenase inhibitory activity of the ethanol fraction of *A. carambola* L. leaves from Assam and to analyze the correlation between the total flavonoid and phenol contents based on IC_{50} values.

MATERIALS AND METHODS:

Collection and authentication of the plant:

The *Averrhoa carambola* leaves, from the Oxalidaceae family, were gathered from A Block in Baksa, Assam. The plant was identified and authenticated by Dr. Souravjyoti Borah, an Assistant Professor in the Department of Botany at Gauhati University, Assam.

Plant extraction [9]:

The *Averrhoa carambola* leaves were air-dried in the laboratory and ground into a coarse powder. The plant material was then subjected to continuous extraction using the hot Soxhlet extraction method. The initial extraction was performed with 500 mL of petroleum ether as the solvent, lasting for 48 hours. The resulting extract was collected, and the solvent was evaporated using a rotary vacuum evaporator at 40°C. Following the petroleum ether extraction, the remaining plant residue (marc) was further extracted with 500 mL of 95% ethanol for another 48 hours. The ethanol extract obtained was then collected and concentrated by evaporation at 40°C using a rotary vacuum evaporator.

Total Phenol and Flavonoid Content Estimation [10]:

The total phenolic content will be assessed following the method described by Siddhuraju and Becker (2003). Triplicate 50 µL samples of extracts, prepared in their respective organic solvents at mg/mL concentrations, will be transferred into test tubes, and the volume will be adjusted to 1 mL with distilled water. In tube 1, sequential additions will include 0.5 mL of Folin-Ciocalteu phenol reagent (diluted 1:1 with water) and 2.5 mL of a 20% sodium carbonate solution. The mixture will then be vortexed and incubated in the dark for 40 minutes. After incubation, the absorbance will be measured using a spectrophotometer at a wavelength of 725 nm against a reagent blank. The total phenolic content will be expressed in terms of gallic acid equivalents (GAE).

The flavonoid content of the extracts will be determined using the method outlined by Zhishen et al. (1999). For each extract, 500 µL (1 mg/mL in its respective organic solvent) will be placed in

test tubes along with 2 mL of distilled water. A blank sample will be prepared using 2.5 mL of distilled water. To both the test samples and blank, 150 µL of 5% $NaNO_2$ solution will be added, followed by incubation at room temperature for 6 minutes. After this, 150 µL of 10% $AlCl_3$ solution will be added to the test tubes and blank, with another 6-minute incubation period. Subsequently, 2 mL of 4% NaOH solution will be introduced to all tubes, and the final volume will be adjusted to 5 mL with distilled water. The mixtures will be vortexed thoroughly and left to stand at room temperature for 15 minutes. The intensity of the resulting pink coloration, indicative of flavonoid presence, will be measured using a UV spectrophotometer at 510 nm. Rutin will serve as the standard for flavonoid quantification, and the results will be expressed as Rutin equivalents (RE). All experiments will be performed in triplicate.

***In-vitro* anti-inflammatory assay:**

In accordance with established protocols, one assay method was employed to evaluate the anti-inflammatory activity of the extracts *in-vitro*.

Lipoxygenase activity inhibitor test [11]:

Anti-lipoxygenase activity is evaluated using linoleic acid as the substrate and lipoxygenase as the enzyme. Either soybean lipoxygenase or human recombinant lipoxygenase can be utilized for this assay. The procedure for analyzing this activity is summarized as the reaction mixture consists of 160 µL of 100 mM sodium phosphate buffer (pH 8.0), 10 µL of plant extract at varying concentrations (10, 25, 50, 100, and 200 µg/mL), and 20 µL of soybean lipoxygenase solution (167 U/mL). The mixture is then incubated at 25°C for 10 minutes. The reaction is initiated by adding 10 µL of sodium linoleic acid solution as the substrate. Absorbance is measured at 234 nm at intervals of one minute over a total duration of three minutes using a UV-Vis spectrophotometer.

Aspirin is used as a positive reference drug, while a control sample is prepared by excluding the plant extract or drug from the reaction mixture. All experiments are conducted in triplicate. The percentage of inhibition is calculated using the following formula: % Inhibition = [(A control-A sample) /A control] x 100

Where 'A sample' is the absorbance of a sample solution, and 'A control' is the absorbance of the control solution.

RESULTS AND DISCUSSION:

Results of total phenolic content:

Numerous studies have highlighted the importance of phenolic compounds in human health due to their antioxidant properties. The total phenolic content in the ethanolic extract of *Averrhoa carambola* was determined to be 8.323 mg of gallic acid equivalents per gram of extract. This was calculated using the regression equation ($y = 0.0084x + 0.0856$; $R^2 = 0.97$). A standard calibration curve was generated at 765 nm using known concentrations of gallic acid, as illustrated in Fig. 1.

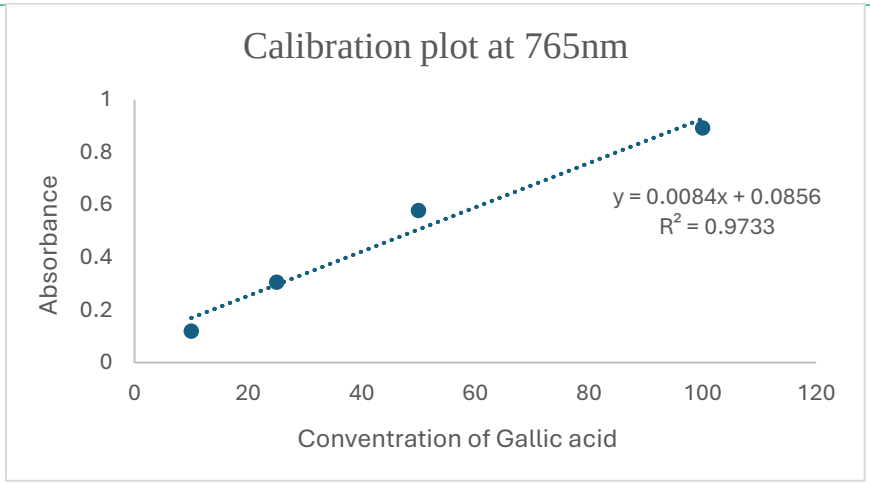

Figure 1: Calibration plot for phenolic determination

Results of total flavonoid content:

Flavonoids are among the most significant natural phenols and are recognized for their diversity and abundance among natural compounds. Using the calibration curve [y = 0.0102x - 0.0637 (R^2 = 0.9693)], the analysis revealed a substantial flavonoid content in the ethanolic extract, measuring 1.52 mg of quercetin equivalents per gram of extract.

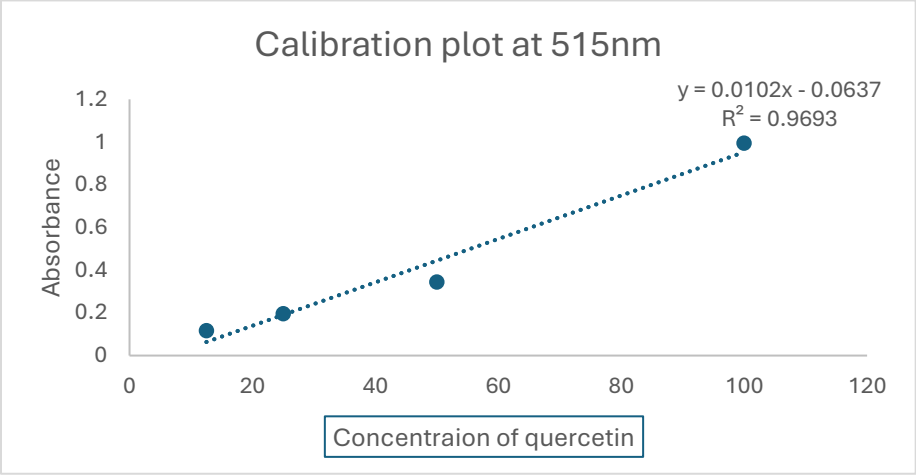

Figure 2: Calibration plot for flavonoid determination.

Results of Lipoxygenase activity inhibitor test:

The development of new in vitro test systems has encouraged the screening of plants to identify potential leads for drug discovery. The plant lipoxygenase pathway shares significant similarities with the arachidonic acid cascade in animals. Therefore, in vitro lipoxygenase inhibition serves as an effective model for identifying plants with anti-inflammatory properties. Lipoxygenases (LOXs) are highly sensitive to antioxidants, and their inhibitory effect is largely due to the suppression of lipid hydroperoxide formation. This occurs through the scavenging of lipidoxy or lipid peroxy radicals, which are generated during enzymatic peroxidation. Consequently, this process reduces the availability of lipid hydroperoxide substrates required for the LOX catalytic cycle. The ethanol extract was tested at concentrations of 10, 25, 50, 100, and 200 µg/mL, demonstrating anti-lipoxygenase inhibition of 28.89%, 35.47%, 41.22%, 50.67%, and 72.46%, respectively. The IC50 value of the ethanol extract was determined to be 96.86 µg/mL, with the highest inhibition observed at 200 µg/mL. In comparison, the standard drug aspirin exhibited 79.44% inhibition at 200 µg/mL, with an IC50 value of 43.86 µg/mL. These findings indicate that the ethanol extract possesses notable anti-inflammatory potential by inhibiting lipoxygenase enzyme activity. This suggests that the plant's ethanol extract could be valuable for research on inflammation and its associated physiological conditions, including aging, cancer, neurological disorders, and other related diseases.

Table 1: Effect of ethanol plant extract on lipoxygenase inhibitory action

Sample	Concentration (µg/ml)	% Inhibition (µg/ml)	IC_{50} (µg/ml)
Averrhoa carambola	10	28.89 ±0.08	96.86
	25	35.47 ±0.05	

	50	41.22±0.06	
	100	50.67±0.05	
	200	72.46±0.04	
Aspirin	10	42.04±0.06	43.86
	25	46.97±0.03	
	50	52.32±0.04	
	100	63.00±0.05	
	200	79.44±0.05	
Absorbance of the control: 0.811. Each test was carried out triplicate (n=3)			

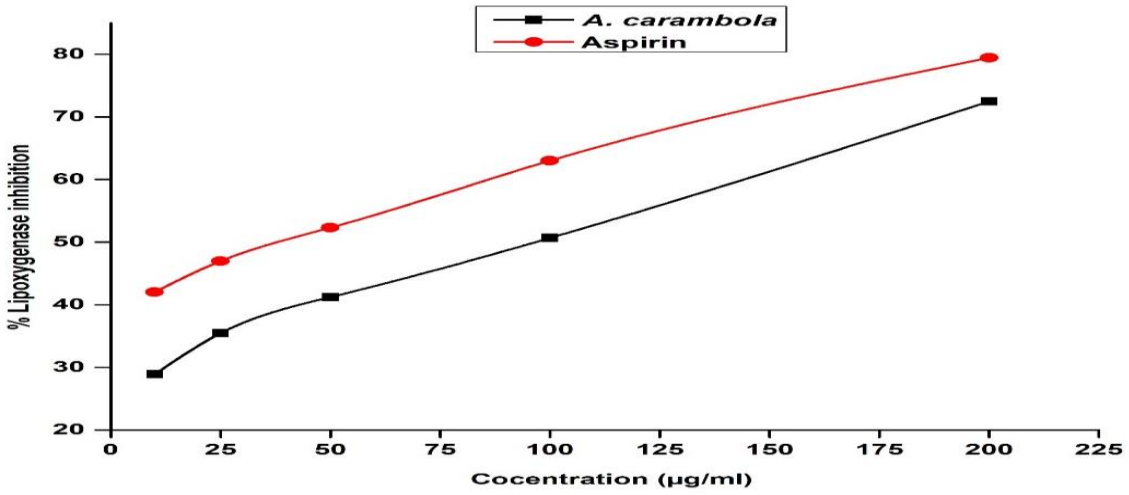

Figure 3: Effect of ethanol plant extract of *A. carambola* and aspirin on lipoxygenase action

CONCLUSIONS:

The total flavonoid content in the leaf extracts showed a strong inverse correlation with IC_{50} values, with a correlation coefficient of −0.701. Similarly, the total phenolic content in the ethanol fraction was also inversely correlated with IC_{50} values, with a correlation coefficient of −0.603. These

findings indicate that as the total flavonoid and phenolic content increases, the IC_{50} values decrease, suggesting greater lipoxygenase inhibition.

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