



EVALUATION OF *IN VITRO* ANTI-INFLAMMATORY ACTIVITY OF FRUIT EXTRACTS OF *SPONDIAS MOMBIN*

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Abstract

Spondias mombin (family: Anacardaceae) is a fruit producing tree commonly found in India, Nigeria, Sri Lanka, Brazil and those of other tropical regions. It is commonly called as 'Hog plum' or 'Yellow mombin'. It is traditionally used as diuretic, febrifuge and for the treatment of various stomach ailments. This study seeks to appraise the *in vitro* anti-inflammatory potential out of the ethanol and aqueous fruit extracts. Inhibition of protein denaturation method and Membrane stabilization test by heat induced haemolytic method was used to carry out *in vitro* anti-inflammatory activity. Protein denaturation inhibition was evaluated by Bovine serum albumin and Egg albumin method. These were estimated at concentration range of 50 µg/ml-300 µg/ml. Both the extracts showed concentration dependent protein denaturation inhibition. The percentage inhibition of membrane stabilization was increased with increase in the concentration of fruit extracts. The current study suggests that the ethanol and aqueous extracts of *Spondias mombin* have significant anti-inflammatory activity with standard drug diclofenac.

Key words: *Spondias mombin*, anti-inflammatory, bovine serum albumin, egg albumin.

Introduction

Inflammation is defence or protective mechanism of body, which involves immune cells, blood vessels and molecular mediators (Joseph, L. *et al.*, 2016). When the body cells faces stress from various agents like physical agents, chemical agents and microbes, it will result in inflammation. It is marked by redness, swelling, heat, pain and loss of affected area function. The cells release various kinds of mediators like prostaglandins, kinin and histamines when the cells become damaged. These mediators will increase vasodilation and permeability of capillaries (Ullah, H.A. *et al.*, 2014). Inflammation will increase protein denaturation and causes some alterations in the membrane (Padmanabhan, P. *et al.*, 2012).

Non-steroidal anti-inflammatory drugs are extensively used for the treatment and managing inflammation but they cause number of side effects. Hence there is a need for identifying a safer anti-inflammatory agent. The study of plants of different species which are used traditionally for the treatment of inflammation plays an important role in finding a safer anti-inflammatory agent. The prime

advantage of using herbal medicine is their recognizable efficacy and marginal lateral impacts.

Spondias mombin (family: Anacardaceae) is a fruit producing tree commonly available in Nigeria, Brazil, India, Sri Lanka and other tropical regions. It is generally called as 'Hog plum' or 'Yellow mombin'. It is a deciduous tree to the level of 15-22m height and 1.5m in diameter. The bark is thick, corky and deeply fissured. The branches are low and are glabrous with pinnate, oblong and broadly acuminate leaves. From January to May the flowers blossom and are sweet scented, with small white flowers in large, lax terminal panicles. The fruits hang on the tree in numerous clusters exceeding a dozen. Quite rich in vitamin B1 and vitamin C, the fruit is mainly an oval seed. The seed has oil content. (Ayoka, A.O. *et al.*, 2008). Fruit is normally yellow colored when ripe and has a leathery coat with pulp layer inside (Oladimeji, A.O. *et al.*, 2016). The decoction of bark and leaves were traditionally used for emesis, for the treatment of diarrhea, hemorrhoids, gonorrhoea and leucorrhoea. The fruit decoction is used as diuretic and febrifuge. A tea of leaves and flowers is used to get relieve from stomachache (Asuquo, O.R. *et al.*, 2013).

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Materials and Methods

Collection of Plant material and Extraction

The fruits of *Spondias mombin* were picked from in and around the Mangalore. The fruits were cleaned, dried and powdered. The powdered fruits were subjected for extraction. The ethanol extract was prepared by maceration method and aqueous extract was prepared by infusion method. The extracts thus obtained were subjected for *in vitro* screening of anti-inflammatory activity.

In vitro screening of Anti-inflammatory Activity

• Protein denaturation inhibition method

Bovine serum albumin method: One percent aqueous solution of bovine serum albumin was prepared. The reaction mixture pH (6.3) was adjusted with 1N hydrochloric acid. The reaction mixture consisting of 0.45ml of bovine serum albumin solution and various concentrations of fruit extracts were incubated 20 minutes at 37°C and heated for 5 minutes at 51°C. 2.5ml of phosphate buffer was added, after the samples were cooled. The absorbance was measured at 660nm. Instead of test extract double distilled water was added for the control (Leelaprakash, G. et al., 2011). Diclofenac sodium was taken as a standard. The protein denaturation inhibition percentage was determined by following formula.

$$\% \text{ inhibition} = \frac{Abs_{control} - Abs_{test}}{Abs_{control}} \times 100$$

Where $Abs_{control}$ is the control absorbance and Abs_{test} is the test absorbance.

Egg albumin method: To 0.2ml egg albumin and 2.8ml phosphate buffer was added. To this solution 2ml of varying concentration of test extract was added. For the control, double distilled water was added instead of test extract. The resulting reaction mixture was incubated at 37°C for 15 minutes and afterwards heated to 70°C for 5 minutes. The absorbance was taken at 660nm, after cooling (Fernandes, J. et al., 2017). Diclofenac sodium was taken as a standard. The protein denaturation inhibition percentage was determined by following formula.

$$\% \text{ inhibition} = \frac{Abs_{control} - Abs_{test}}{Abs_{control}} \times 100$$

Where $Abs_{control}$ is the control absorbance and Abs_{test} is the test absorbance

Membrane stabilization test by Heat induced haemolytic method

Preparation of suspension of red blood cells (RBCs):

Table 1: Percentage inhibition of Bovine serum albumin denaturation by fruit extracts of *Spondias mombin*.

Concentration (µg/ml)	% Inhibition of BSA denaturation ±SEM		
	Standard	Ethanol Extract	Aqueous Extract
50	16.26±0.347	13.25±0.347	14.45±0.347
100	25.90±0.347	21.08±0.347	23.49±0.347
150	40.16±0.200	34.93±0.347	39.75±0.347
200	50.60±0.347	43.37±0.347	44.37±0.200
250	66.86±0.347	62.65±0.347	61.24±0.200
300	75.30±0.347	71.68±0.347	71.68±0.347

Fresh human blood was extracted and moved to the centrifuge tubes. The tubes were centrifuged for 10 minutes at 3000rpm and washed three times with equal amount of normal saline. The blood volume was measured and reconstituted with normal saline as 10 percent v/v suspension.

Heat induced haemolytic method: The reaction mixture consists of 1ml of test sample solution and 1 ml of 10 percent RBCs suspension, instead of test sample, only saline has been added to the control test tube. For 30 min, all the centrifuge tubes containing reaction mixture were incubated in water bath at a temperature of 56°C. The tubes were cooled under running tap water, at the end of the incubation. The reaction mixture was centrifuged for 5 minutes at 2500 rpm and the supernatants absorbance was taken at 560 nm. The experiment was carried out in triplicates for all the test extract samples. Percentage membrane stabilization activity was calculated (Govindappa, M. et al., 2011).

Results and Discussion

Preliminary phytochemical screening of the fruit extracts of *Spondias mombin* was carried out to find the presence of various phytoconstituents. The preliminary phytochemical studies of the fruit extracts indicated the existence of reducing sugars, flavanoids, triterpenoids, glycosides, resins, saponins, steroids, tannins.

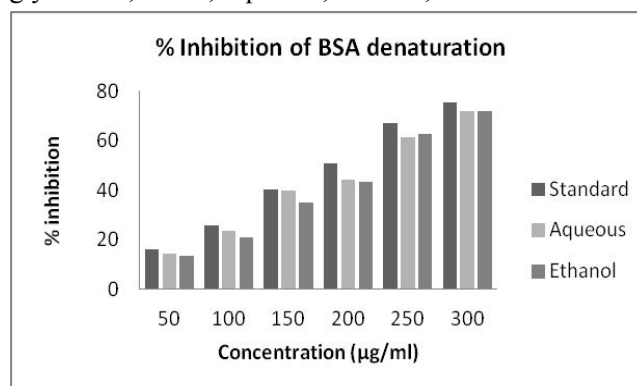


Fig. 1: Effect of fruit extracts and Diclofenac sodium on Bovine serum albumin denaturation.

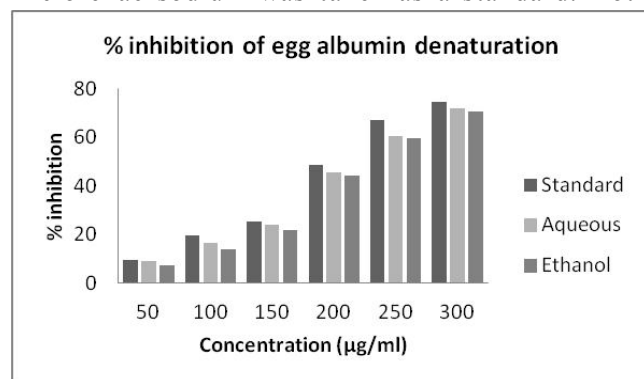
Table 2: Percentage inhibition of Egg albumin denaturation by fruit extracts of *Spondias mombin*.

Concentration (µg/ml)	% Inhibition Egg albumin denaturation ±SEM		
	Standard	Ethanol Extract	Aqueous Extract
50	9.49±0.098	7.32±0.098	8.93±0.162
100	19.46±0.197	14.05±0.162	16.40±0.197
150	25.44±0.129	21.71±0.197	23.99±0.194
200	48.69±0.098	44.28±0.112	45.62±0.194
250	67.11±0.162	59.34±0.134	60.23±0.261
300	74.66±0.129	70.32±0.134	71.63±0.194

In this study ethanol and aqueous extract of fruits of *Spondias mombin* were subjected to *in vitro* anti-inflammatory studies by egg albumin denaturation method and bovine serum albumin method. Proteins such as egg albumin and milk casein are soluble in water in their normal form. These structures are damaged through a variety of processes like heat acids, strong alkalis, urea, salicylate, alcohol and ultraviolet light etc. The molecules in a denatured protein will take a staggering number of different forms. One of the characteristics of many non-steroidal anti-inflammatory drugs has been documented to be their capacity to stabilize heat-treated albumin at physiological pH.

The result of anti-inflammatory activity by bovine serum albumin method was given in the table 1. Both ethanol and aqueous extracts showed concentration dependent inhibition of protein denaturation and they showed substantial inhibitory effect. Here diclofenac was used as standard anti-inflammatory drug. And both the fruit extracts showed comparable results with standard. Both aqueous and ethanol extracts showed same percentage of inhibition at concentration 300µg/ml and that was 71.68%. Least percentage of inhibition of protein denaturation was observed at 50µg/ml.

The result for the anti-inflammatory activity by egg albumin denaturation method was given in the table 2. Diclofenac sodium was taken as a standard. Both

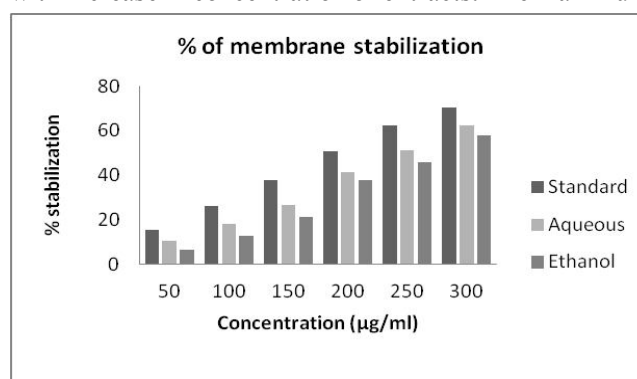
**Fig. 2:** Effect of fruit extracts and Diclofenac sodium on egg albumin denaturation.**Table 3:** Percentage of membrane stabilization by fruit extracts of *Spondias mombin*.

Concentration (µg/ml)	% of membrane stabilization ± SEM		
	Standard	Ethanol Extract	Aqueous Extract
50	15.45±0.241	6.76±0.241	10.38±0.241
100	26.08±0.418	13.04±0.418	18.35±0.241
150	37.92±0.241	21.25±0.241	26.81±0.418
200	50.72±0.418	37.68±0.418	41.30±0.418
250	62.56±0.241	45.65±0.418	51.20±0.241
300	70.53±0.241	57.97±0.418	62.31±0.418

aqueous and ethanol extracts demonstrated concentration dependent inhibition of egg albumin denaturation. The aqueous extract showed comparatively good results than ethanol extract. The maximum percentage inhibition of protein denaturation was observed at 300µg/ml. The maximum percentage inhibition by aqueous and ethanol fruit extract was 71.63% and 70.32% respectively. Least percentage inhibition of protein denaturation was 8.93% and 7.32% by aqueous and ethanol extract respectively at concentration 50µg/ml.

The HRBC membrane stabilisation was also used as a tool to evaluate the *in vitro* anti-inflammatory activity as the erythrocyte membrane is comparable to the lysosomal membrane. Lysosomal stabilisation is effective in reducing the inflammatory response by blocking the liberation of activated neutrophil's lysosomal components, which trigger more inflammation of the tissue. Non-steroidal medications show their action either by suppressing the enzymes of lysosome or by stabilizing the lysosomal membrane.

The results of Heat induced haemolytic method was given in the table 3. The percentage of membrane stabilization was increased with increase in the concentration of fruit extracts. The minimum membrane stabilization was observed at concentration 50µg/ml. There was a gradual increase in membrane stabilization with increase in concentration of extracts. The maximum

**Fig. 3:** Effect of fruit extracts and Diclofenac sodium on membrane stabilization.

percentage inhibition shown by aqueous and ethanol fruit extract was 62.31% and 57.97% respectively which was observed at concentration 300µg/ml.

Conclusion

In vitro anti-inflammatory activity of the fruits of *Spondias mombin* was evaluated by using standard methods like protein denaturation method using bovine serum albumin fraction and egg albumin; and by HRBC membrane stabilization method. The aqueous extract as well as the ethanolic extract of the *Spondias mombin* exhibited significant percentage inhibition when compared to the standard. Diclofenac sodium was used as the standard drug. The *in vitro* anti-inflammatory activity showed positive outcomes, thereby encouraging their usage in inflammation management.

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