



# PHYTOCHEMICAL EVALUATION AND ANTINFLAMMATORY ACTIVITY OF SEED EXTRACT OF *ANNONA SQUAMOSA*

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## ABSTRACT

The present study was aimed to evaluate the anti-inflammatory properties of seed extracts of *Annona squamosa* in rats. The ethanol extract of *Annona squamosa* was extracted by using Soxhlet extraction apparatus. The preliminary phytochemical studies revealed for the presence of alkaloids, reducing sugar, fixed oil and fats, phenolic compounds and tannins, proteins and amino acids. The anti-inflammatory profile of this extracts was investigated on the basis of paw edema induced by carrageenan. The extract (100 mg/kg) caused a maximum inhibitory activity on compared with standard reference drug indomethacin. The ethanolic seed extract of *A. squamosa* significantly ( $p < 0.001$ ) inhibits the inflammation of about 36.33% in paw edema induced by carrageenan and indomethacin (10 mg/kg) inhibits about 35.13%. On the basis of these findings, it may be inferred that *A. squamosa* exerts anti-inflammatory activity at the second phase of carrageenan inflammation.

**Key words:** *Annona squamosa*, Seed extract, Phytochemical studies, Anti-inflammatory activity, Carrageenan.

## INTRODUCTION

*Annona squamosa* belongs to the family of *annonaceae* and is native of India. A large, evergreen, straggling shrub or small tree found wild and cultivated in various parts. Bark thin, grey; leaves oblong-lanceolate or elliptic, flowers solitary leafs opposed or 1-4 on short extra axillary branchless, pedicles 12-19 mm leaves and fruits used against tumours. Leaf extract used as insecticidal, spasmogenic and spasmolytic. Leaf juice is used in the treatment of animal wounds and paste applied for ulcers. Seeds are used against emetic and diuretics in rats. It has anti-fertility activity also in albino mice. Bark is

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powerful astringent and tonic. Root is violent purgative, unripe and dried fruits are used in diarrhoea and dysentery. Leaves and tender stem of the plant contains anonaine, roemerine, norcorydine, corydine, norlaurcline, isocarydine, norisocarydine glaucine and nitro heteroside. Essential oil from leaves contains an alkaloid carvone. Fruits contain folic acid and peel oil (0.1%) contain turpenes, alpha and beta-pinenes and limonine. Roots and bark contains alkaloids anonaine, corydine, isocorbine, glaucine, beta-sitosterol, camphor and bornol<sup>1-5</sup>. The availability of seeds is much and collection of plant material is easy. Based on the above facts, the seeds of *Annona squamosa* are selected for the study. This study reports the effects of extract of *Annona squamosa* seeds against inflammation caused by carrageenan in Wister albino rats.

## EXPERIMENTAL

### Preparation of extract

The seeds of *Annona squamosa* were collected in Tiruchirappalli district where the plant is cultivated under natural condition and authenticated the same. The seeds of *Annona squamosa* were dried at shade at room temperature, pulverized by a mechanical grinder and sieved through 40 meshes. The powdered materials were extracted with ethanol using Soxhlet extraction apparatus. The extract was concentrated under reduced pressure. The ethanol free semi-solid mass, thus obtained, was used for phytochemical studies and anti-inflammatory activities.

### Animals

Albino rats (Wister Strain) of either sex weighing between 150-200 g B.W were used. They were individually housed under standard environmental conditions ( $25 \pm 3^\circ\text{C}$  and light-dark cycles). Animals were used after one week of acclimatization in the departmental animal room. Animals were fed with rat feed and water ad libitum. The animals were divided into groups of three in six animals each and fasted for 12 hours before the experiment. The study is approved by IAEC.

### Phytochemical analysis

Ethanol seed extracts of *A. squamosa* were listed to determine the various phytochemical groups using standard methods<sup>6,7</sup>. The anti-inflammatory properties of the plants may be attributed to the secondary metabolites present in it.

### Anti-inflammatory activity<sup>8</sup>

The anti-inflammatory activity was studied by carrageenan induced hind paw edema method<sup>9-11</sup>. The dose of drugs administered to the different experimental groups, like control (Group 1) received 0.5 mL of normal saline, standard (Group 2) received indomethacin (10 mg/kg body weight) and test (Group 3) received seed extract of *A. squamosa* (100 mg/kg body weight) by subcutaneously. 5% w/v of acacia mucilage was used as a vehicle at a dose of 5 mL/kg. The drugs were given, 1 hour prior to the study. In all the groups, the inflammation was induced by single sub planter injection of 0.1 mL of freshly prepared 1%w/v carrageenan solution in normal saline<sup>9-12</sup>. Before injection of carrageenan, the average volume ( $V_0$ ) of the left hind paw of each rat was calculated from three readings that did not deviate more than 3%. After injection of the carrageenan, readings ( $V_t$ ) were obtained for each rat at the end of the zero minutes<sup>13</sup>, 60, 120, 180, 240, 360 and 480 min with the aid of a plethysmometer. The edema was expressed as an increase in the volume of paw, and the percentage of inhibition of inflammation<sup>11-14</sup> for each rat and each group was calculated by new bould method<sup>15</sup>.

### Statistical analysis

All values are presented as mean  $\pm$  SEM. The percentage variation was evaluated for each group. Test of significance was statistically evaluated by student's t-test method<sup>16,17</sup>.

## RESULTS AND DISCUSSION

The seeds of *A. squamosa* were selected to study the anti-inflammatory activity. The extraction process was carried out with ethanol using Soxhlet extraction apparatus after pulverizing and sieving through 40 mesh. Then it was dried under reduced pressure. Its phytochemical evaluation reveals the presence of alkaloids, reducing sugar, fixed oil and fat, phenolic compounds and tannins, proteins and amino acids. The effects of extract of *A. squamosa* on paw edema induced by carrageenan are shown in the Table 1.

The seed extract showed (100 mg/kg) significant inhibition of the edema. Edema, which develops after the carrageenan administration, is a biphasic event<sup>1</sup>. The initial phase is attributed to the release of histamine and serotonin. The edema maintained between the first and the second phase is due to kinnin like substances<sup>18</sup>. The second phase is said to be promoted by the prostroglandin like substances. Carrageenan induces paw edema by inducing protein rich exudates containing a large number of neutrophils<sup>19</sup>. It has been reported that the second phase of edema is sensitive to drugs like hydrocortisone,

phenylbutazone and indomethacin<sup>20</sup>. Indomethacin is a non-selective cyclooxygenase inhibitor<sup>21</sup>. Thus, the investigated seed extract of *A. squamosa* exerts anti-inflammatory activity at the second phase of carrageenan inflammation. The results are significant ( $p < 0.001$ ) as shown in Table 1 and are comparable to standard indomethacin. These evidences for the anti-inflammatory study verify objective of the present study. In this study, we concluded that the seed extract of *A. squamosa* is having good anti-inflammatory activity.

**Table 1: Data showing the anti-inflammatory activity of seed extract of *Annona squamosa***

Groups	% Increase in paw volume ( Mean $\pm$ S.E., n=6)							
	Post insult time of assay in minutes							
	0	60	120	180	240	360	480	% Inhibition of Paw volume
Group 1 (Control)	65.5 $\pm$ 5.4	72.2 $\pm$ 6.4	84.9 $\pm$ 5.9	99.6 $\pm$ 8.9	109.0 $\pm$ 7.3	108.6 $\pm$ 7.7	108.6 $\pm$ 6.9	NIL
Group 2 (Indomethacin 10 mg/kg)	62.2 $\pm$ 3.9	66.6 $\pm$ 3.8	72.8 $\pm$ 3.4	70.5 $\pm$ 4.1*	70.7 $\pm$ 2.8*	71.5 $\pm$ 4.4	70.5 $\pm$ 5.6	35.13
Group 3 ( <i>A. squa</i> 100 mg/kg)	50.6 $\pm$ 2.2	58.6 $\pm$ 3.9	67.8 $\pm$ 2.9	68.6 $\pm$ 2.4*	69.4 $\pm$ 4.8*	72.0 $\pm$ 5.4	66.9 $\pm$ 3.9	36.33

All results expressed as Mean  $\pm$  SEM from six observations \* $p < 0.001$  Vs Control

## ACKNOWLEDGEMENT

We are thankful to Dr. K. Ruckmani, Principal, Periyar College of Pharmaceutical Sciences for Women, Trichy for providing working facilities and to Dr. A. Jaswanth for his constant guidance in proceeding with this work.

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*Accepted* : 14.03.2008