

# Evaluation of acute anti-inflammatory activity of *Psidium guajava* leaf extracts in Wistar albino rats

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## ABSTRACT

*Psidium guajava* L. (Myrtaceae), commonly known as guava in English, is an evergreen small tree grown in tropics and subtropics and also cultivated commercially in India for its consumable fruits. The present study assessed two defatted successive solvent (ethyl acetate and methanol) extracts of *P. guajava* leaf for their acute anti-inflammatory potential by carrageenan induced hind paw oedema in Wistar albino rats. Both the test extracts exhibited significant and comparable anti-inflammatory activity. The methanol extract was found to be a bit more active than the ethyl acetate extract. The present preliminary study demonstrated promising acute anti-inflammatory activity of *P. guajava* leaf in Wistar rats.

**Key words:** Anti-inflammatory, *Psidium guajava*, oedema, leaf, carrageenan.

## Introduction

Inflammation is a pathophysiological response to injury, infection or destruction characterized by heat, redness, pain, swelling and disturbed functions. Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. It is the body response to inactivate or destroy the invading organisms, to remove the irritants and set the stage for tissue repair. It is triggered by the release of chemical mediators from injured tissue and migrating cells. [1] The most commonly used drug for management of inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs), which have several adverse effects especially gastric irritation leading to formation of gastric ulcers. [2, 3]

Natural products have contributed significantly towards the development of modern medicine. Recently traditional medicine worldwide is being re-evaluated by extensive research on different plant species and their active therapeutic principles. The rich wealth of plant kingdom can represent a novel

source of newer compounds with significant anti-inflammatory activities. The major merits of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects and low cost.

*Psidium guajava* L. (Myrtaceae), an evergreen small tree, commonly known as guava in English, is an important food crop and medicinal plant indigenous to South America and grown in tropical and subtropical countries. It is widely used as food and in folk medicine around of the world. It is commercially cultivated in India for its consumable and palatable fruits. A number of metabolites in good yield and some have been shown to possess useful biological activities belonging mainly to phenolic compounds, flavonoids, carotenoids, terpenoids and triterpenes. Extracts and secondary metabolites of this plant, particularly those from leaves and fruits possess useful pharmacological activities. *P. guajava* is mainly known for its antispasmodic and antimicrobial properties in the treatment of diarrhoea and dysentery. It has also been used extensively as an oral hypoglycaemic agent. Several pharmacological studies have demonstrated the ability of this plant to exhibit antioxidant, hepatoprotection, anti-allergic, antimicrobial, antigenotoxic, antiparasitic, cytotoxic, antispasmodic, cardioactive, antitussive, antidiabetic, anti-inflammatory and anti-nociceptive activities, supporting its traditional uses. [4-6] However, the

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acute anti-inflammatory assessment of defatted ethyl acetate and methanol extracts from *P. guajava* leaf are still not reported. Therefore, in the present investigation we attempted these studies on the leaf extracts of *P. guajava* grown in India.

## Materials and Methods

**Plant material:** The mature leaves of *Psidium guajava* L. (Myrtaceae), were collected during November 2012 from Kalyani, Nadia, West Bengal, India. The plant material was taxonomically identified at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India. The voucher specimen (CNH/6/2013/Tech.II/964) was maintained in our research laboratory for future reference. The plant material was shade-dried with occasional shifting and then powdered with mechanical grinder, passing through sieve no. 40, and stored in an air-tight container.

**Preparation of plant extracts:** The dried powdered material (350 g) was defatted with petroleum ether (60-80°C), the percentage extractive value was 0.81% w/w. The defatted powdered material thus obtained was further extracted with ethyl acetate and methanol for 72 h in a percolator. The solvent was distilled off in reduced pressure and resulting semisolid mass was vacuum dried using rotary flash evaporator to yield a solid residue and the percentage extractive values were accordingly 3.78% w/w and 20.27% w/w respectively. The preliminary phytochemical analysis was performed for all three extracts to identify the phytoconstituents present in the extracts. [7]

**Drugs and chemicals:** Indomethacin and  $\lambda$ -carrageenan were from Sigma-Aldrich Chemical Corp. (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade obtained commercially.

**Experimental animals:** Adult Wistar rats of Wistar strain weighing  $200 \pm 20$  g were procured from registered breeders (Reeta Ghosh & Co., Kolkata, India) and maintained under standard laboratory conditions (temperature  $25 \pm 2^\circ\text{C}$  with dark and light circle 14/10 h). They were allowed free access to

standard dry pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The mice were acclimatized to laboratory condition for 10 days before commencement of the experiment. All experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee (Reg. no. 367001/C/ CPCSEA).

## Anti-inflammatory evaluation: Carrageenan-induced rat paw oedema:

The overnight fasted rats were divided into four groups ( $n = 6$ ). The first group (which served as control) received normal saline (5 ml/kg body wt., p.o.). The second and third groups received the ethyl acetate and methanol extracts at the doses of 200 mg/kg b.w., p.o. respectively. The fourth group (which served as reference) received indomethacin (10 mg/kg body wt., p.o.). After 30 mins, acute inflammation was produced by the subplantar administration of 0.1 ml of 1 % (w/v) of freshly prepared suspension of  $\lambda$ -carrageenan in the right hind paw of each rat. The paw volume was measured at 0 h and after 1 h intervals up to 4 h after carrageenan challenge by using a plethysmometer (Ugo Basile, Italy). The difference between the two readings was taken as the volume of oedema and the percentage of inhibition was calculated with respect to control and expressed the percentage of protection by using the following formula: [8]

$$\left( \frac{\text{Control mean} - \text{Treated mean}}{\text{Control mean}} \right) \times 100 \%$$

**Statistical analysis:** The data are represented as mean  $\pm$  standard error of mean (SEM). Degree of significance was assessed by Student's 't' test. *P* values less than 0.001 were considered as statistically significant.

## Results and Discussion

Preliminary phytochemical studies showed the presence of steroids in the petroleum ether extract; triterpenoids, alkaloids and phenolic compounds in the ethyl acetate extract; and steroids, alkaloids,

phenolic compounds, glycosides and carbohydrates in the methanol extract.

The present study establishes the significant acute anti-inflammatory activity of *P. guajava* leaf extracts in experimentally induced acute inflammation in Wistar rats. The inflammatory response can be readily produced in the form paw oedema with the help of irritants or phlogistic agents. Such agents like carrageenan, formalin, bradykinin, histamine, serotonin etc when injected into the dorsum of the foot of the rats they produce acute paw oedema within a few minutes of injection. Carrageenan induced rat paw oedema has been most commonly used as an ideal experimental animal model for acute inflammation. [9, 10]

Carrageenan-induced acute inflammatory oedema is generally believed to be a biphasic response. The early phase (1-2 h) of the carrageenan model is mainly mediated by histamine and serotonin (5-HT). The late phase (2-4 h) is mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages [11]. In the present study, both the test extracts produced significant inhibition of carrageenan induced rat paw oedema after a period of 4 h (Table 1). This indicates the two test extracts were active in both the early and late phases of carrageenan induced acute hind paw

inflammation in rats. The methanol extract was found to be slightly more active than the ethyl acetate extract.

Preliminary phytochemical studies revealed presence of phenolic compounds in both the test extracts. Polyphenolic compounds are putative natural products that are known to several important biological activities including anti-inflammatory properties [12]. The polyphenols content may be responsible for its anti-inflammatory action against carrageenan-induced acute inflammation in albino rats.

Based on the results obtained from the present preliminary study, it can be concluded that both the defatted ethyl acetate and methanol extracts of *Psidium guajava* leaf possessed comparably effective acute anti-inflammatory actions in Wistar albino rats. Further studies are presently necessary to confirm the identity of the bioactive principles responsible for these actions.

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**Table 1:** Effect of *P. guajava* leaf extracts against carrageenan induced rat paw oedema.

Treatments	1 h	2 h	3 h	4 h	% Inhibition
Normal control	0.73±0.08	1.40±0.57	1.80±0.57	1.66±0.08	-
Ethyl acetate extract (200 mg/kg)	0.32±0.05*	0.53±0.06*	0.41±0.04*	0.28±0.07*	83.13
Methanol extract (200 mg/kg)	0.27±0.03*	0.42±0.04*	0.32±0.04*	0.24±0.06*	85.54
Indomethacin (10 mg/kg)	0.20±0.05*	0.50±0.05*	0.36±0.03*	0.23±0.03*	86.14

Values are mean ± SEM (*n* = 6). \**p* < 0.001 when compared with normal control

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