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Evaluation of *In Vitro* Anti-Inflammatory Potential of *Psidium guajava* Seeds Extract

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ABSTRACT

Secondary metabolites obtained from medicinal plants are progressively used in the treatment of various diseases or pathogenic conditions as complementary medicine. Inflammation is a pathological condition including wide range of diseases such as arthritis, osteoarthritis, inflammatory bowel disease, chronic asthma etc. The present study reveals the efficiency of *Psidium guajava* Linn. (*Myrtaceae*) seeds extract for anti inflammatory activity by simple, non toxic, less time consuming and reliable HRBC membrane stabilization method, as it is similar to lysosomal membrane which influence inflammation process. The main constituents of guava seeds possess glycosides, carotenoids and phenolic compounds. Furthermore, seeds and peel are treated as wastes by the food processing industry and are toss out, so their use may decrease the discard of these parts of guava as waste. The resent study reveals the estimation of *in-vitro* anti-inflammatory potential of guava seed extract on the basis of traditionally use of Guava in gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, cough, sore throat, inflamed gums, and a number of other conditions.

INTRODUCTION:

Psidium guajava Linn. is popularly known as guava (family Myrtaceae) has been used traditionally as a medicinal plant for different ailments in all over the world. There are two familiar varieties of guava: the red (*P. guajava* var. *pomifera*) and the white (*P. guajava* var. *pyrifera*) [1, 2]. Leaves, pulp and seeds are used to get rid from respiratory and gastrointestinal disorders, and as an antispasmodic, anti-inflammatory, as a cough sedative, anti-diarrheic, in the management of hypertension, obesity and in the control of diabetes mellitus. It also possesses anticancer properties [3]. The seeds are used as antimicrobial, gastrointestinal, anti-allergic and anticarcinogenic activity [4-5].

Inflammation is part of the complex biological response shown by complement system that ensures survival during infection and tissue injury. It is also a protective attempt by the organism to remove the injurious stimuli and initiate the healing process [6]. Inflammation can be categories as acute or chronic inflammation. Acute inflammation is the initial response of the body to deleterious stimuli and is achieved by increased movement of plasma and leukocytes from the blood into the injured tissues. The process of acute inflammation is initiated by cells already present in the tissues. This is characterized by marked vascular changes, including vasodilatation and maximizes capillary permeability which is instigated by the actions of the various inflammatory mediators [7]. Chronic inflammation is a drag out inflammatory response that leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissues from the inflammatory process [8].

MATERIALS AND METHODS:

Collection of Plant Materials: The fruits of *Psidium guajava* were collected from college campus of Kamla Nehru College of Pharmacy, Butibori, Nagpur Maharashtra. Plant material was identified and authenticated in the department of Botany, RTM Nagpur University Nagpur, Maharashtra. The collected materials were cleaned and flesh was removed for further processes of separation of seeds and extraction.

Preparation of the Extract: Seeds were taken (100g) and subjected to alcoholic extraction (Soxhlet Extraction) Then, it was allowed to evaporate excess of solvent under rotary vacuum evaporator until clear liquid (Oil) is obtained. Then the oil extract was then re-dissolved in ethanol at 1 mg/ml ratio and used for evaluating *in-vitro* anti-inflammatory potentials.

Table No. 1: Phytochemical Screening of seed extract of *Psidium guajava*

Chemical Test	Inference
Alkaloids	-
Carbohydrates	-
Glycosides	+
Flavonoid	-
Tannin	+
Terpenoids	+
Oil and fats	+
Steroids	-

(-) indicates absent; (+) indicates present

***In-vitro* anti-inflammatory activity:**

HRBC Membrane Stabilization: Human red blood cells (HRBC) suspension was prepared according to the previously described method [9]. The blood was collected from healthy human volunteers who have not taken any NSAIDs for 2 weeks prior to the experiment and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of blood was measured and re-constituted as 10% v/v suspension with normal saline. Hypotonicity induced haemolysis was used for membrane stabilization assay. The reaction mixture (4.5 ml) consisted of 2 ml hypotonic saline (0.25% NaCl), 1 ml of 0.15 M phosphate buffer (pH 7.4), 1 ml extract (200, 400, 600, 800 and 1000 µg/ml) in normal saline and 0.5 ml of 10% human RBC in normal saline. In blank 1 ml of isotonic saline was used instead of extract while control was devoid of red blood cells. The mixtures were incubated at 50^{o}C for 30 min. The tubes were cooled under running tap water for 20 min followed by centrifugation at 1500 rpm for 10 min. Absorbance of the supernatant was read at 560 nm.

Membrane stabilization was calculated by using the formula:

(Abs of blank - Abs of extract) / Abs of control* x 100.

*The control represents 100%

Drugs interacts with membrane hence the model used in this study is Erythrocytes membrane stabilization [10-11]. NSAIDs stabilize erythrocytes against stress hemolysis. Moreover, they prevent the release of hemoglobin as a result of their membrane stabilizing activity [12]. The human red blood cells (HRBC) model is selected to assess the anti-inflammatory activity of *Psidium guajava*. In this study, 05 different concentrations of the aqueous extract of *Psidium guajava* seed extract have been evaluated for their HRBC membrane stabilization activity. High concentration (1000 µg/ml) of the fruit flesh extract was found to stabilize the HRBC membrane up to 66.47% (Table 2), which is comparable to the activity of the standard analgesic Aspirin (57.71%). All the extracts exhibited membrane stabilization activity in a dose-dependent manner.

Table No. 2: HRBC membrane stabilization activity of the seeds extract of *Psidium guajava*

Concentration (µg/ml) Stabilization (%)	Concentration (µg/ml) Stabilization (%)
62.5	46.32±0.16
125	48.28±0.26
250	59.15±0.71
500	61.36±0.98
1000	66.47±0.43
Aspirin (100µg/ml)	57.71±0.58

CONCLUSION:

Various researches on the medicinal use of plant extract is must in modern science as many chemically synthesized drugs are highly effective in causing many toxic effects in humans. The phytochemical and pharmacological investigations carried out on *Psidium* seeds extract validate the immense potential of this plant in the treatment of inflammation. The inhibition is dose dependent with high doses of the extract showing a significance percentage of inhibition. The anti-inflammatory effects exhibited by the extracts could as well be attributed to other phytochemicals present in the seeds. Past studies have shown that phytochemicals including tannins possess analgesic and anti-inflammatory activities [13]. To better understand the mechanisms by which phytochemicals of *Psidium guajava* seeds extract act, further studies are warranted. Additional researches are needed for the compound isolation and identification for the product development from *Psidium guajava* seeds for future gen-

erations. Further, the present study suggests that *Psidium guajava* could serve as a lead in the development of a novel herbal anti-inflammatory agent and every medicinal property of many medicinal plants are also to be determined.

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