

Anti-Inflammatory Activity of Various Fractions of Methanolic Extract of *Punica granatum* rind with its Phytochemical Evaluation

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Abstract

Objective: To evaluate anti-inflammatory potential of rind extract of *Punica granatum* by *in-vitro* anti-inflammatory model and its phytochemical evaluation.

Method: The fractionation of methanolic extract of *Punica granatum* rind was done using various solvents and evaluated for anti-inflammatory activity using *in-vitro* human red blood cell (HRBC) membrane stabilization method and its phytochemical study.

Results: The *in-vitro* membrane stabilizing test showed that butanol, n-hexane and aqueous extracts have potent anti-inflammatory activity which were 42.66%, 14.1% and 28% protection, respectively as compared to Standard drug. The potency of the rind extracts of PG were compared with standard diclofenac in stabilization test and which showed 81.76% protection in *in-vitro* HRBC membrane stabilization method. The extract showed the dose dependent anti-inflammatory activity.

Conclusions: The present investigation has confirmed the anti-inflammatory activity of fractions of methanolic extract of *Punica granatum* rind due to presence of alkaloids, steroids, terpenoids for the first time and provided the pharmacological evidence in the favour of traditional uses of *Punica granatum* as an anti-inflammatory agent.

Keywords- *Punica granatum*, Anti-inflammatory activity, HRBC membrane stabilization method.

Introduction

Nowadays more light is being shed on the importance of medicinal plants, many of which have always been used as mere traditional or folk remedies, nonetheless today, they are being studied and analyzed for potential biological activities that will thus explain why the locals have always used them for treating various diseases and illnesses¹. The dried rind of fruit is used in the treatment of amoebic dysentery, diarrhoea, and is a specific remedy of tapeworm infestation and certain inflammatory condition². Pomegranate has been used for centuries to confer health benefits in a number of inflammatory diseases. Based on its usage in Ayurvedic and Unani medicine, dietary supplements containing pomegranate extract are becoming popular in the Western world for the treatment and prevention of arthritis and other inflammatory diseases³.

Inflammation is a reaction to an infection, irritation or foreign substance. It is a part of the host defense mechanisms. It is known to be involved in the inflammatory reactions such as release of histamine, bradykinin, prostaglandins, fluid extravasations, cell migration, tissue breakdown and repair which are aimed at host defense and usually activated in most disease condition. Edema formation, leukocyte infiltration and granuloma formation are main manifestations of inflammation⁴.

The inflammatory response changes with time and can be divided into phases. The rapid phase occurs within seconds to minutes and consists of vasodilation; increased blood flow, edema, and pain. The acute phase is characterized by induction of inflammatory genes by NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells) and other transcription factors. During this phase, moderate amounts of inflammatory mediators are produced. The chronic phase occurs over months to years and is marked by dramatically increased production of inflammatory mediators. The secondary chronic phase of inflammation occurs after years of oxidative damage has degraded blood vessels and tissues. Such chronic inflammation appears to play a role in many disease states, such as arteriosclerosis and cancer⁵.

The present research aims to investigate in-vitro anti-inflammatory activities of various fraction of methanolic extracts of leaf of *Punica granatum* rind.

Material and Methods

Plant Material

The fruit of *Punica granatum* were collected from local market of Udaipur in April 2012. The fruit were identified by from Department of Horticulture, Rajasthan College of Agriculture, Udaipur, India. Rind portion of fruit was separated out from the fruit. The rind were dried under the shed and powdered into the coarse form. It was successively extracted with methanol using soxhlet apparatus. The extract was concentrated under reduced

pressure and was preserved and the residues were then successively partitioned between water and n-hexane followed by chloroform and n-butanol. The solutions were completely evaporated to give the respective fractions.

In-vitro Anti-Inflammatory Activity

It was tested by Human red blood cell (RBC) membrane stabilization method. The blood was collected from healthy human volunteer who had not taken any NSAIDs for 2 weeks prior to the experiment and was mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3 000 rpm. The packed cells were washed with isosaline and a 10% suspension was made from it. Hydroalcoholic extract was prepared (100,200, 400, 800 and 1600 μ g/mL), respectively using distilled water and to each concentration 1 m of phosphate buffer, 2 m hyposaline and 0.5 m of HRBC suspension were added. It was incubated at 37^oC for 30 min and centrifuged at 3000 rpm for 20 min. The hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac (50, 100,200, 400, 800 and 1600 μ g/mL) was used as reference standard and a control was prepared by omitting the extracts. The percentage of HRBC membrane stabilization or protection was calculated by using the following formula⁴⁻⁸:

$$\text{Percent Protection} = \frac{100 - [\text{O.D of Sample}]}{\text{O.D of Control}} * 100$$

Preliminary Phytochemical Analysis

The preliminary phytochemical analysis of the fractions of methanolic extract of *Punica granatum* rind was conducted for the detection of alkaloids, flavonoids, tannins/phenolic compounds, steroids, terpenoids. The phytochemical screening of the fractions of methanolic extract were performed as per described methods⁹⁻¹⁰.

A. Detection of alkaloids

- **Mayer's Test:** The extracts were treated with Mayer's reagent (Potassium mercuric iodide). The formation of a yellow cream precipitate indicated the presence of alkaloids.
- **Dragendroff's Test:** The extracts were treated with Dragendroff's reagent (solution of Potassium bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

B. Detection of carbohydrate

- **Molisch's Test:** The extracts were treated with 2 drops of alcoholic α -naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrate.

C. Detection of saponins

- **Foam Test:** The extracts were shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

D. Detection of flavonoids

- **Shinoda Test:** The extracts were treated with few fragments of magnesium metal separately followed by drop wise addition of concentrated hydrochloric acid. The formation of magenta colour indicated the presence of flavonoids⁴.
- **Alkaline reagent test:** Crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids⁵.

E. Detection of steroid

- **Salkowski's test:** Crude extract was mixed with 2ml of chloroform. Then 2ml of concentrated H₂SO₄ was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring, i.e., glycone portion of the glycoside.

F. Detection for phenols and tannins

- The extracts were mixed with 2ml of 2% solution of FeCl₃. A blue-green or black coloration indicated the presence of phenols and tannins,

G. Detection for terpenoids

- The extracts were dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H₂SO₄ was added and heated for about 2 minutes. A grayish colour indicated the presence of terpenoids

H. Detection of protein

- **Millon's test:** Crude extract when mixed with 2ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein

RESULTS

The preliminary qualitative phytochemical screening of the methanolic fractions of pomegranate rind extract revealed the presence of alkaloids, flavonoids, carbohydrates, tannins, sterols and triterpenes.

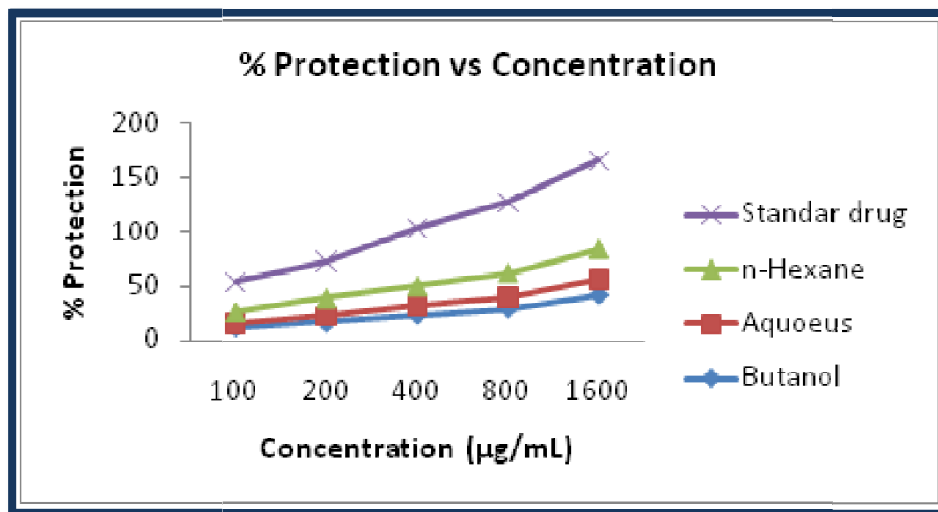
The result of the human red blood cell membrane stabilization test were shown in Table 1. The aqueous, butanol and n-hexane showed a concentration

dependent anti inflammatory activity, and the protection percent increased with increase in the concentration of the drug. Where as the choloroform did not showed any potent anti-inflammatory activity.

Among all the extracts, butanolic fraction showed the highest percent protection. All the results were compared with diclofenac (standard which showed 81.76 % protection)

Table.1 Anti inflammatory activity of fractions of methanolic extracts of *Punica granatum* rind membrane stabilization by HRBC method.

S. No	% Protection				
	Conc μ /ml.	Butanolic fraction	n-hexane fraction	Aqueous fraction	Diclofenac
1	100	12.34 \pm 0.01	3.8 \pm 0.00	10.41 \pm 0.01	28.4 \pm 0.02
2	200	17.16 \pm 0.10	5.57 \pm 0.02	16.16 \pm 0.00	34.75 \pm 0.05
3	400	24.33 \pm 0.01	8.5 \pm 0.02	17.25 \pm 0.03	54.41 \pm 0.03
4	800	30.16 \pm 0.01	9.5 \pm 0.01	23 \pm 0.01	65.67 \pm 0.02
5	1600	42.66 \pm 0.02	14.1 \pm 0.01	28 \pm 0.01	81.76 \pm 0.01



Graph 1: Anti inflammatory activity of fractions of methanolic extracts of *Punica granatum* rind membrane stabilization by HRBC method.

Table.2 Preliminary phytochemicals screening of fractions of methanolic extracts of *Punica granatum*

S. No.	Tests	Aqueous fraction	Butanolic fraction	Cloroform fraction	n-Hexane
1.	Alkaloids				
	Mayers test	-ve	+ve	-ve	-ve
	Dragendarff's	-ve	+ve	-ve	-ve
2.	Carbohydrate				
	Molisch test	+ve	-ve	+ve	+ve
3.	Flavonoids				
	Shinoda test	-ve	-ve	-ve	-ve
	Alkaline reagent test	+ve	+ve	-ve	+ve
4.	Tannins/ Phenolic compounds				
	Ferric chloride test	+ve	+ve	-ve	-ve
5.	Steroids				
	Salkowski test	+ve	+ve	-ve	-ve
6.	Irioid glycosides	-ve	-ve	-ve	-ve
7.	Terpenoid	-ve	+ve	+ve	+ve
8.	Saponin				
	Foam test	-ve	-ve	-ve	-ve
9.	Protein				
	Millon's test	-ve	-ve	-ve	-ve

Discussion¹¹

The pharmacological properties of the rind extract resembles those of the non-steroidal anti-inflammatory drugs which are known to possess anti-inflammatory activity. One of the major mechanism involved in the anti-inflammatory activity of NSAIDs is due to inhibition of prostaglandin biosynthesis. But in the present study of in vitro evaluation of anti-inflammatory property, because the erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release¹¹. According to the table 1 the result indicated that the fractions of rind extract of *Punica granatum* shows dose dependent anti-inflammatory activity when it compared to the standard drug

The chemical responsible for the pharmacological activity are not known. By the study of various phytochemical tests, it was found that steroid flavonoids, carbohydrates, tannis, sterols and triterpenes were present in fractions. Anti-inflammatory activity of the plant part might be shown due to presence of these phytoconstituents.

In further study, isolation of lead compound which is responsible for the activity is in progress to develop a better remedy for the inflammation.

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