Research Article

Anti-inflammatory activity of *Punica granatum* peel extract – An *in vitro* analysis

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ABSTRACT

Aim: The aim of this study was to evaluate the anti-inflammatory activity of *Punica granatum* peel extract by *in vitro* analysis. **Objective:** The purpose of the study is to evaluate the anti-inflammatory activity of *P. granatum* peel extract by *in vitro* analysis. **Materials and Methods:** *P. granatum* was collected from local market, shade dried, and peeled. The ethanol extraction of *P. granatum* peel powder was done as per the standard method. Different concentrations of the extract were used for anti-inflammatory activity by inhibition of albumin denaturation and proteinase inhibitory action methods. All samples were analyzed in triplicate. The results were statistically analyzed. **Results:** The results indicate that the methanol extracts of *P. granatum* possess anti-inflammatory properties. **Conclusion:** It is concluded from the present study that methanol extracts of *P. granatum* possess anti-inflammatory properties due to the strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids, and phenols in the plant extracts.

KEY WORDS: Anti-inflammatory, *Punica granatum*, Plant extracts, Albumin denaturation

INTRODUCTION

In recent years, the field of natural product biology, ethnomedicine, as well as biodiversity prospecting approaches, has received renewed attention in recent times.[1,2] Herbal products and natural remedies used in ancient traditional medicine have been a vital source for medically beneficial drugs. *Punica granatum* is the botanical name for pomegranate, and it is a small fruit-bearing tree or shrub that grows between 5 and 8 meters (16–26 feet) tall. It is a widely found common fruit in Iran and the Mediterranean region, which is commonly used for therapeutic formulae, cosmetics, and food seasoning.

In India, it has been used in traditional medicine for the treatment of various infectious and inflammatory diseases. The unripe fruit reduces inflammation and is used in the treatment of keratitis and also as a tonic to increase appetite.[3] The ripe fruit is used as a laxative, astringent, and diuretic and used in bronchitis, chest troubles, and earache. The juice of the fruit is administered to cure inflammation of liver, emesis, edema, cough, leprosy, and anorexia.[4] The fruit rind and bark are consumed orally to prevent diarrhea, dysentery, piles, bronchitis, and bilious complications. The decoction of a well-dried rind of the fruit is taken for the relief of dysentery and stomachache. The flower buds are powdered and given internally for the relief of bronchitis, diarrhea, and dysentery of children. A decoction of the flowers is gargled to reduced oral and throat inflammation.[5] The biological activities such as antibacterial,[6] antifungal,[7] anthelmintic,[8] antifertility,[9] antioxidant,[10] antidiabetic,[11] antiatherogenic,[12] and anti-ulcer[13] of the various extracts of different parts of this plant have also been reported.

Inflammation is the first natural, physiological defense system in the human body that protects against injuries caused by physical wounds and poisons. This defense system is also called as short-term inflammation that can destroy infectious microorganisms, eliminate irritants, and maintain normal physiological functions. However, long-term overinflammation might cause dysfunctions of the regular physiology. In this study,

Access this article online

Website: jprsolutions.info | ISSN: 0975-7619

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Received on: 12-11-2018; Revised on: 17-01-2019; Accepted on: 05-02-2019

the *in vitro* anti-inflammatory activity of *P. granatum* peel extract will be assessed using alpha-amylase and alpha-glucosidase.

According to recent reports, *P. granatum* is rich in polyphenols, including mainly ellagitannins, gallotannins (punicalin, punicalagin, pedunculagin, punigluconin, granatin B, and tellimagrandin I),[14] and anthocyanins (delphinidin, cyanidin, and pelargonidin).[10] It is also reported to contain alkaloids, flavonoids, and hydrolyzable tannins (such as punicalin, pedunculagin, punicalagin, gallic, and ellagic acid esters of glucose) which possess strong anti-inflammatory and antioxidant properties.[15]

MATERIALS AND METHODS

Plant Material

Fresh fruits of *P. granatum* (Punicaceae) were purchased from the local market in Chennai, India.

Extraction

Fruit peels (1 kg) of *P. granatum* were dried in the shadow in air draft and comminuted to powder and exhaustively extracted under reflux over a boiling water bath by 2 L of an ethanol/bidistilled water (3:1) mixture for 8 h. The extract was filtered and the process was repeated 3 times. The solvent was removed under reduced pressure at 45°C. Finally, the process yielded 100 g of a sticky dark brown material.

Assessment of *In Vitro* Anti-Inflammatory Activity of Peel Extract of *P. granatum*

Inhibition of albumin denaturation by P. granatum

The reaction mixture was consisted of test extracts and 1% aqueous solution of bovine albumin fraction, and the pH of the reaction mixture was adjusted using a small amount of 1N HCl. The sample extracts were incubated at 37°C for 20 min and then heated to 51°C for 20 min; after cooling the samples, the turbidity was measured at 660 nm (Ultraviolet-Visible Spectrophotometer Model 371, Elico India Ltd). The experiment was performed in triplicate. The percentage inhibition of protein denaturation was calculated as follows: Percentage inhibition = (Abs Control –Abs Sample) × 100/Abs control.

Statistical Analysis

The results are expressed as mean ± SD. The difference between experimental groups was compared by one-way analysis of variance followed by Dunnett's multiple comparison test (control vs. test) using the software Graph Pad Instat.

RESULTS

Effect of *P. granatum* Peel Extract on Inhibition of Albumin Denaturation *In Vitro*

[Figure 1 and Table 1] represent the effect of different concentration of *P. granatum* peel ethanolic extract on inhibition of albumin denaturation. Protein denaturation is a well established cause of inflammation. As a part of the investigation on anti-inflammatory activity, ability of different concentration of *P. granatum* peel ethanolic extract to inhibit albumin denaturation was studied. *P. granatum* peel ethanolic extract shows higher inhibiting activity in the higher concentration. Aspirin was used as standard drug.

DISCUSSION

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent, or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, the ability of plant extract to inhibit protein denaturation was studied. It was effective in inhibiting heat-induced albumin denaturation. Maximum inhibition of 69% was observed at 500 µg/ml. Aspirin, a standard anti-inflammatory drug, showed the maximum inhibition of 79% at the concentration of 500 µg/ml compared with control [Table 1].

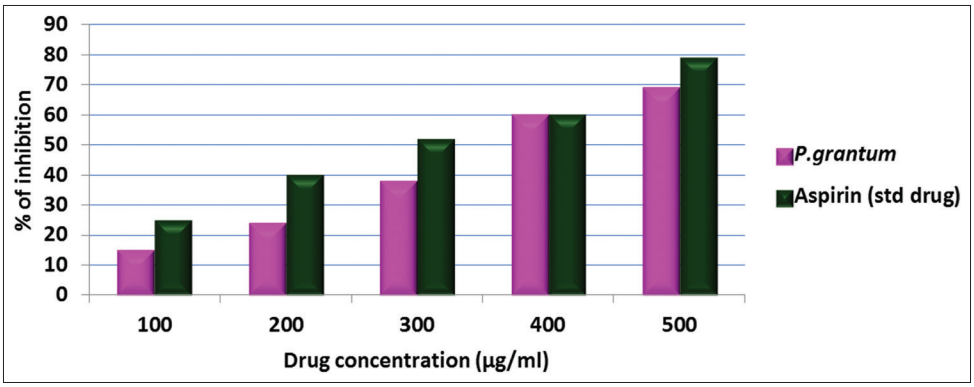

Figure 1: Effect of *P. granatum* peel extract on inhibition of albumin denaturation

Table 1: Effect of different concentration of *P. granatum* peel extract on inhibition of albumin denaturation

Treatment (s) concentration	Concentration (µg/ml) *P. granatum*	% inhibition of albumin denaturation by *P. granatum*	Aspirin (standard drug) (µg/ml) Aspirin	% inhibition of albumin denaturation by Aspirin
P. granatum	100	15	100	25
P. granatum	200	24	200	40
P. granatum	300	38	300	52
P. granatum	400	60	400	60
P. granatum	500	69	500	79

P. granatum: Punica granatum

CONCLUSION

In the present study, results indicate that the methanol extracts of *P. granatum* possess anti-inflammatory properties. These activities may be due to the strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids, and phenols. The extract fractions serve as free radical inhibitors or scavenger or acting possibly as primary oxidants and inhibited the heat-induced albumin denaturation.

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Source of support: Nil; Conflict of interest: None Declared