Trypsin inhibitory activity of *Punica granatum* Linn.

D.S. Samiulla¹, D. Prashanth¹*, A. Amit¹, B.V. Venkataraman²

¹. Bioassay Unit, Research & Development Centre, Natural Remedies Pvt. Ltd., Plot No. 5B, Veerasandra Indl. Area, Hosur Road, Bangalore-561 229, India.
². Department of Pharmacology, St. John’s Medical College, Bangalore - 560 034

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**Abstract**

Four successive extracts (petroleum ether, chloroform, methanol and water) of *P. granatum* fruit rind were tested for *in-vitro* trypsin inhibitory activity using commercially available enzyme (Porcine pancreatic type II trypsin) and substrate (Benzoyl-D,L-arginine-4-nitroanilide) and the activity was compared with standard trypsin inhibitor from hen egg white (ovomucoid). The methanolic extract was found to be most active and the activity was comparable to that of the reference standard.

**Keywords:** *Punica granatum*, Ovomucoid, Trypsin inhibitor, Proteases.

**1. Objective**

The present investigation was carried out to study the four successive extracts (petroleum ether, chloroform, methanol and water) of *P. granatum* fruit rind for *in-vitro* trypsin inhibitory activity.

**2. Plant Material**

*Punica granatum* Linn. (Punicaceae) fruit rind was collected from Bangalore and was authenticated by National Institute of Science Communication, New Delhi, India-Voucher specimen (BAU/AM/03) is preserved in our pharmacognosy department.

**3. Preparation of extracts**

Successive extract of petroleum ether, chloroform, methanol and water were prepared using soxhlet apparatus. (yields: 0.78, 0.2, 30 and 15% w/w respectively on dry basis). Preliminary phytochemical screening gave positive test for tannins and alkaloids [1-2].

**4. Tested activity**

**In-vitro Trypsin inhibition assay**

This was done by the method of Cannell *et al* [3]. In brief, 400 µl of 0.4 M Tris-HCl pH 7.5; 400 µl of enzyme solution (Sigma, Porcine pancreatic type II trypsin, 150 units/ml in 50 mM Tris-HCl buffer pH 7.5); 800 µl of test solution or reference standard (ovomucoid, Fluka) were incubated at 37°C for 30 minutes and then 800 µl of substrate solution (Sigma, Benzoyl-D, L-arginine-4-nitroanilide, 43.3 mg dissolved in 1 ml of dimethyl sulphoxide then made up to 100 ml with 50 mM Tris-HCl buffer pH 7.5) was added. The absorbance was read immediately...