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Bioassay Report

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Trypsin inhibitory activity of *Punica granatum* Linn.

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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 upto 100 ml with 50 mM Tris-HCl buffer pH 7.5) was added. The absorbance was read immediately

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