

**Abstract No: MO-29**

**Evaluation of the effect of oxidizing and reducing agents on trypsin inhibitory activity of *Vigna mungo* seeds (Black Gram)**

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Trypsin is one of the widely distributed serine proteases in living organisms, which is involved in vital physiological functions. In spite of its involvement in survival of the organisms, it was reported that trypsin is associated in pathogenesis of different type of human cancers. Therefore, trypsin inhibitory proteins have been gained attention as a potential treatment strategy against such cancers. Seeds of legumes have been recognized as potential natural resources of trypsin inhibitory proteins. Black gram (*Vigna mungo*) is a popular legume cultivated in Sri Lanka and Field Crops Research and Development Institute of Sri Lanka (FCRDI) has released a novel breed of black gram called Anuradha. The attempts were made to assess trypsin inhibitory activity (TIA) of its seeds and characterization of the active trypsin inhibitory proteins for future discovery of anticancer agents. The objectives of the present study was to evaluate the effect of oxidizing and reducing agents on the activity of trypsin inhibitory proteins present in seed of the local variety and to investigate the ionic nature of active proteins using ion exchange chromatography. Fresh mature seeds of variety Anuradha were collected from FCRDI and the crude protein extract (20%) of milled seeds was prepared using distilled water. The TIA of the extract was assessed by the method explained by Kunitz (1947) with slight modifications. To evaluate the effect of oxidizing and reducing agents on TIA, the seed extract was incubated with hydrogen peroxide (1-2%, v/v), dimethyl sulphoxide (1-5%, v/v) and varying concentrations of  $\beta$ -mercaptoethanol for 30 minutes, followed by the measurement of TIA. To discover the ionic nature of the active proteins, the crude protein extract was fractionated by ion exchange chromatography using positively charged Diethylaminoethyl cellulose as the stationary phase. The column was washed with phosphate buffer (pH 7.4) and then eluted with 1 M NaCl. The absorbance of each eluted fraction was measured at 280 nm. The TIA was assessed for the fractions with highest absorbance values. The TIA activity of the crude protein extract was  $73.21 \pm 0.29\%$ . In the presence of the oxidizing agents, dimethyl sulfoxide and hydrogen peroxide the TIA of the test extract was  $59.91 \pm 0.69\%$  and  $27.08 \pm 0.34\%$  respectively. The activity of seed samples incubated with 0.1 M, 0.5 M, 1 M and 2 M of  $\beta$ -mercaptoethanol was  $70.72 \pm 0.23\%$ ,  $58.63 \pm 0.53\%$ ,  $46.31 \pm 0.72\%$  and  $39.47 \pm 0.44\%$  respectively. Ion exchange chromatography resulted one fraction with high protein content at pH 7.4 which exhibited  $3.12 \pm 0.62\%$  of TIA. The TIA of the washing fraction was  $35.46 \pm 0.08\%$ . The results of the present study revealed that Dimethyl sulfoxide decreased the TIA in a moderate level while hydrogen peroxide caused a drastic decrease. Increasing concentration of the reducing agent  $\beta$ -mercaptoethanol gradually decreased the TIA of the test sample. The observations of column chromatography indicated that the seed sample of black gram contain active trypsin inhibitory proteins which exist in an anionic or neutral form in pH 7.4.

**Keywords:** Trypsin inhibitory activity, Black Gram, *Vigna mungo*, oxidizing agents, reducing agents