ISSN: 2659-2029
Proceedings of the 5th Research Symposium of the Faculty of Allied Health Sciences
University of Ruhuna, Galle, Sri Lanka
August 26, 2022

PP 24

Effect of Temperature on Cysteine Inhibitory Activity of Seed Extracts of *Vigna unguiculate* (Cowpea) Sri Lankan Breeds

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Background: Disturbances in regulation of cysteine activity may leads to severe disease conditions such as cancers, neurodegenerative disorders and cardiovascular diseases. Therefore, the discovery of natural cysteine inhibitors paved the way to a novel therapeutic strategy against such diseases.

Objectives: To investigate the cysteine inhibitory activity (CIA) of seed extracts of two local breeds of *Vigna unguiculate* (cowpea) and the effect of temperature on CIA.

Methods: Seeds of two local breeds known as Dahawala and Bombay were collected from the Field Crops Research and Development Institute of Sri Lanka. A concentration gradient (1.25, 2.5, 5, 10, and 20, % w/v) of aqueous seed extracts was screened for CIA. The seed extracts were incubated with L-cysteine in phosphoric acid buffer (pH 7.5) and then the casein was added as the substrate. Following the incubation at 37ºC, trichloroacetic acid was added. The absorbance of the supernatant was recorded at 280 nm and percentage cysteine inhibitory activity (CIA%) was calculated. The thermal stability of the inhibitors was studied by pre-incubation of seed sample in different temperatures following evaluation of CIA.

Results: Among the concentrations screened, the maximum CIA% was exhibited by 10% seed extracts of both breeds [Bombay, 67.5 (±0.01%); Dahawala, 65.5 (±0.03%)]. Hence, 10% extract was assessed for thermal stability. Dahawala seed extract demonstrated its maximum CIA% at 37ºC [69.52 (±0.03%)], while it was 39.44 (±0.07%) at 60ºC. The extract did not exert any activity beyond 80ºC. Bombay seeds extract showed the optimum CIA% at 37ºC [65.27 (±0.06%)], while the inhibitory activity at 60, 80 and 100ºC were 32.63 (±0.02%), 26.60 (±0.08%) and 18.03 (±0.01%), respectively.

Conclusions: The results revealed that, the seed extracts of both breeds contain a considerable amount of cysteine inhibitors. The inhibitors present in Dahawala seeds are heat-labile, while the inhibitors in Bombay seeds are heat stable as they demonstrated a considerable activity even at high temperatures.

Keywords: *Cysteine inhibitors, Legumes, Proteases, Vigna unguiculate*