UNIVERSITI PUTRA MALAYSIA

PURIFICATION, CHARACTERISATION AND INHIBITION STUDIES OF PROTEASE FROM *CORIANDRUM SATIVUM*

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By

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Faculty: Faculty of Biotechnology and Biomolecular Sciences

Protease from coriander leaf (*Coriandrum sativum*) was evaluated for its ability to detect selected heavy metals using Bradford-protease-casein assay system. Considering the highly polluted environment with heavy metals contributed by industrial wastages and its implications on public health, this present study was dedicated to provide a rapid and sensitive assay for the detection of heavy metals in the environmental samples. The basis of the protein assay using casein as a substrate relies upon the inability of the Bradford reagent to stain polypeptide with less than molecular weight of 2 kDa. Casein that has been stained by the Bradford reagent gives a dark blue color. However, the degradation product is not stained by the reagent and the solution remains brown in color. In the presence of heavy metals that inhibit protease activity, casein would remain undigested and the color would remain blue even after incubation. Optimization studies were carried out for this protease prior to heavy metals inhibition studies. The optimization studies include enzyme concentration, substrate concentration, pH, temperature and time of incubation. The

optimum concentration of protease, substrate, temperature and incubation time for protease were 0.45 mg/ml protease, 0.43 mg/ml casein, 35^{o}C and 20 min respectively after a period of heavy metals incubation. This enzyme was then purified through anion exchanger using DEAE- Cellulose column and gel filtration using Agilent ZORBAX column. The molecular weight detected was around 55 kDa. Protease activity obtained from coriander was found to be optimum at pH around 8 to 9.5. For this bioassay, two heavy metals showed inhibition towards enzyme activity at a concentration of 1 mg/l. The inhibition shown by the heavy metals on protease activity were around 40% for mercury and 70% for zinc. The IC_{50} values of mercury and zinc were 3.22 mg/l and 0.73 mg/l respectively. The limits of detection (LOD) for mercury and zinc were 0.24 mg/l and 0.23 mg/l respectively. The limits of quantitation (LOQ) for mercury and zinc were 0.80 mg/l and 0.76 mg/l respectively. This bioassay using coriander protease was found not to be sensitive towards pesticides and xenobiotics. The advantage of the protease bioassay compared to other bioassay relies on its rapidity, simplicity, economical value, stability in severe conditions such as pH and temperature as well as relatively interference free from detergents, solvents and pesticides.

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I certify that a Thesis Examination Committee has met on 15 May 2011 to conduct the final examination of Baskaran A/L Gunasekaran on his thesis entitled "**Purification, Characterization and Inhibition Studies of Protease from *Coriandrum sativum***" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Masters of Science.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which has been duly acknowledged. I also declare that it has not been previously, and not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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BASKARAN GUNASEKARAN

Date: 16 May 2011

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