

CHAPTER I



INTRODUCTION

Legume seeds are valuable agricultural and commercial crops that serve as important nutrient sources for both humans and animals.⁽¹⁻³⁾ Legume seeds also contain antinutritional compounds such as the protease inhibitors.^(4,5) Protease inhibitors have shown biological effects ranging from antinutritional to beneficial.⁽⁶⁻⁸⁾ Pea seeds have protease inhibitors, with antifungal activity, which are secreted during germination, indicating a protective role of inhibitors.⁽⁹⁾ The inhibitors are known for their roles in response to abiotic^(10,11) and biotic stresses, especially in plant defense processes against insect pest attack,⁽¹²⁾ where they are effective against insect digestive enzymes.⁽¹³⁻¹⁶⁾

Pithecellobium dulce Benth. or *P. dulce* (Ma Kam Thet) in Leguminosae family is a woody legume native in the northwestern regions of Mexico.⁽¹⁷⁾ It is widely distributed throughout India, South East Asia (Thailand, Malaysia, and Indonesia) and can also be found in South Africa and Australia.^(1,18) This tree species has been used for fencing, tanning, feed, and food.^(1,2) Due to their high protein, dietary fiber, and unsaturated fatty acids, seeds of *Pithecellobium* species are traditionally used worldwide for food and feedstuff.^(3,4,18,19) Protein inhibitory activity has been registered for the seeds of several *Pithecellobium* species. However, the majority of protein components in *P. dulce* seeds have not been studied and as such there is a considerable lack of information of the protein composition of *P. dulce* seeds and their biological activities. Large-scale protein analysis (proteomics) has been successfully used to identify and characterize proteins expressed in an organism or cell.^(20,21) It commonly involves the use of two-dimensional gel electrophoresis to separate proteins for quantitative comparison and mass spectrometry for protein identification through database search.⁽²²⁻²⁴⁾ It has been successfully used to

characterize the proteomes of *Arabidopsis thaliana*, *Glycine max*, and *Medicago truncatula*, a model legume.⁽²⁵⁻³⁰⁾ Therefore, it is interesting to characterize of the protein profile of *P. dulce* seeds using two-dimensional gel electrophoresis and tandem mass spectrometry. The identification of proteins from this plant that have no genome and proteome information was done by the combination of *de novo* sequencing and similarity searching. This protein profile can be used to gain a large amount of information about the individual proteins involved in specific biological responses.⁽³¹⁻³⁴⁾ Moreover, it is interesting to study on *P. dulce* seeds which is inhibitory to plant pathogens using chromatography and electrophoresis techniques for protein separation and mass spectrometer for protein identification. The observation of this purified protein will suggest an important role in constitutive host defense mechanisms against microbial pathogens. This may contribute to the development of a biological control strategy for agriculture.

The aims of this study are:

- 1) To identify proteins of the *P. dulce* seed that lacks information of sequence genome.
- 2) To identify the purified bioactive protein from *P. dulce* seed.