1.1 Introduction

Proteases have been described in large numbers naturally in animals, plants and microorganisms and have been extensively studied in order to their structures and functional properties (Laskowski and Kato, 1980; Hibbetts *et al.*, 1999). Proteases are known as proteinases, peptidases or proteolytic enzymes and are involved in many beneficial function. Many biological functions including food digestion, lysosom degradation, and signalling cascades rely on proteases (Fear *et al.*, 2006). The proteases are the enzymes which *in vivo* catalyzed the hydrolytic breakdown of proteins into specific peptide fractions and amino acids (Otlewski *et al.*, 1999; Fear *et.al*, 2006; Habib H. and Khalid M.F., 2007).

Proteases are divided into endoproteases and exoproteases where function as regulators to control endogenous enzymes that regulatory activity of proteolytic, whether the target enzymes are of exogenous or endogenous origin in the presence of the active enzyme (Barret, 1987; Ryan, 1990; Kato, 2002). There are classified according to their mechanism of catalysis and the amino acid essential for its activity, as cysteine proteases with a cysteine, aspartic proteases with an aspartate group, metalloproteases with a metal ions (Zn^{2+}, Ca^{2+} or Mn^{2+}) and serine proteases with serine and histidine (Neurath, 1984; Carlini and Grossi-de-Sa, 2001).

Hans Neurath (1984) was among the first scientist recognized that protease act not only as digestive enzymes, but also fulfill numerous other functions for many biological organism

including food digestion, lysosome degradation, and signaling cascades rely on proteases and also functional for others (Neurath, 1984; Laskowski and Qasim, 1999; Fear *et al.*, 2006). Hans Neurath also recognized that proteinases are highly beneficial and must be extremely controlled by the respective cell or organism because they are potentially hazardous to their natural environment because may lead high pressure on the environment and proteases can be responsible for serious diseases when uncontrolled (Neurath, 1984; Laskowski and Qasim, 1999; Carlini and Grossi-de-Sa, 2001).

The control of proteases is normally achieved by regulated expression, secretion or activation of proproteinases. Proteases also controlled by degradation of mature enzymes and by inhibition of their proteolytic activity (Otlewski *et al.*, 1999). A huge number of inhibitors has been detected, they were isolated from various cells, tissues and organisms (Otlewski *et al.*, 1999). From the experimented, inhibition of proteases by proteins itself has been adaptation to overcome the potential risk of proteolysis and develop specificity of recognition (Otlewski *et al.*, 1999).

The most intensively studied group of protease inhibitors (PI) are serine protease inhibitors such as trypsin, chymotrypsin, elastase and subtilisin (Otlewski *et al.*, 1999). Serine protease inhibitors are divided into subfamilies based on their amino acid sequences (Bode and Huber, 1992). The Kunitz-type and Bowman-Birk families are the best characterized of the serine protease inhibitors.

Trypsin is the largest and diverse serine proteases family that are found in the digestive system of many vertebrates. Once the protease has been activated, trypsins are used as a major form to control on its. In human, trypsin is produced in an inactive form by the pancreases as the inactive proenzyme trypsinogen, where it acts to hydrolyse peptides into their smaller building blocks. These peptides are the result of the enzyme pepsin breaking down the proteins in the stomach. Trypsin enzyme acts to degrade protein and it is often referred to as a proteolytic enzyme or proteinase.

Trypsins are bind with inhibitors which as a competitive substrate analog to form an inactive complex, inactive the digestive enzymes against insect digestive enzymes. The proteolytic activity of the serine protease stops by trypsin inhibitors when its function is no longer necessary. This inhibitor is cause depression of growth, nutritional disorders and pancreatic hypertrophy or hyperplasia (Liener and Kakade, 1980).

The enzymatic mechanism is similar to other serine proteases that contain three residues that is histidine-57, aspartate-102, and serine-195 (Rawling and Barrett, 1994) form a charge relay which serves to make the active site serine nucleophilic. This is achieved by modifying the electrostatic environment of the serine. The enzymatic reaction that trypsins catalyze is thermodynamically favourable but requires significant activation energy. Trypsin also contains an "oxyanion hole" formed by the backbone amide hydrogen atoms of Gly-193 and Ser-195 which serves to stabilize the developing negative charge on the carbonyl oxygen atom of the cleaved amide. In the catalytic pocket (S1) of trypsins are

located the aspartate residue (Asp 189) which responsible for attracting and stabilizing positively-charged lysine or arginine, and is thus responsible for the specificity of the enzyme.

Therefore, in this study research is focused on the function of serine proteases inhibitors by using potential of local plants that are important to control insecticides and thus make them beneficial in agronomical and health relevance. This research study will be involving a through screening, isolation, identification and characterization of protease inhibitors.