CHAPTER 5

CONCLUSION

In this research, proteins from *Parkia spesiosa* were studied. The proteins from the seeds o *Parkia speciosa* were extracted and precipitated by 25%, 40%, 60% and 90% of ammonium sulphate (NH_4SO_4) solution. Advantage of this precipitation is the proteins were not denatured that can be further tested biological activity.

The precipitated proteins by 60% ammonium sulphate were separated by Concanavalin A affinity chromatography (Con A). The bounding fraction on Con A showed hemagglutinating activity with an IC_{50} = 1.17 μg/μl. The unbounding peak (C1) and the bounding peak (C2) were separated by 1-D sodium dodecyl gel electrophoresis. The gel electrophoresis results showed protein profiles. There are two spots in C2 fraction which were C2a, C2b spot and C1a band was the single spot of C1 fraction in coomassie blue staned gel. These proteins have mass about 45, 23 and 40 kDa, respectively. Then, the spots in gel were trypic digested and analyzed by MALDI-TOF MS. and searched by MASCOT program.

The precipitated crude proteins by 25% ammonium sulphate were separated by affi-gel blue gel affinity chromatography. The result showed unbounding peak which were A1, A2 and bounding was A3. A1 and A2 fraction have hemagglutinaing activity but not showed in A3 fraction. So, A1 and A2 were separated by 1-D SDS-PAGE coomassie blue stained gel. The gel results were shown individual separate protein spots. There are two spots Aa and Ab which have molecular weight about 45 and 23 kDa, respectively. After that, the unbounding fractions from affinity column were separated by gel filtration chromatography. From this technique, there are 2 peaks that are Gi and Gj and brought them to define the component of proteins by 1D-SDS PAGE. The gel electrophoresis results were shown protein profiles, Gi fraction appeared two proteins which have similar mass with Aa and Ab. Gj was found the molecular weight by MALDI-TOF MS. which was 21087.96 Da. and showed hemagglutinatins activity with an IC_{50} of 0.39 μg/μl. Consequently, Gi was purified by HPLC and the purified fraction (A1) has ∝-glucosidase inhibition.

Finally, the A1 and Gj fractions were trypic digested and analyzed by MALDI-TOF MS. and searched by MASCOT program.

From amino acid sequence database search, the results of proteins were shown in Table 5.1.

Table 5.1 The conclusion of database search result.

Crude protein % $(NH_4)_2SO_4$	Band of protein	Protein name	Molecular weight	Organism
60%	C1a	Hypothetical protein	20539	*Oryza sativa* (Japonica cultivar-group)
	C2a	Hypothetical protein B1342F01.11	14184	*Oryza sativa* (Japonica cultivar-group)
	C2b	Hypothetical protein	43571	*Arabidopsis thaliana* (Mouse-ear cress).
25%	A1	Hypothetical protein p0466b10.22	55843	*Oryza sativa* (Japonicacultivar group)
	Gj	Putative ristolochene synthase	23232	*Oryza sativa* (Japonicacultivar group)

Suggestion

Putative ristolochene synthase protein might be estimated that it is the lectin as the previous literature in 1992 [21] which was digested to disulphide bond.