CHAPTER IV

CONCLUSION

During the course of research, 10 crude extracts from 3 species of weeds in the family Euphorbiaceae. *E. hirta* L., *E. thymifolia* L. and *E. heterophylla* L. were preliminarily bioassayed with rice (*Oryza sativa* cv RD 23) seedling for plant growth inhibition activity. From the results of crude ethanol extract the stems parts of *E. heterophylla* L. showed 100% inhibition both leaves sheath and root length of rice at 1.0 g/ 3 ml, then the stem part of *E. heterophylla* L. was selected for further investigation.

The extraction and initial fractionation by various organic solvents; *n*-hexane, dichloromethane, ethyl acetate and methanol of the stems part of *E. heterophylla* L. were conducted. Each fraction was subjected to the rice growth inhibition activity. It was observed that the dichloromethane crude extract gave 100% inhibition of both leaf sheath and root length of rice at 1.0 g/ 3 ml, followed by ethanol and hexane crude extracts.

By using bioassay as a guide to search for active compound(s), the dichloromethane crude extract was separated by silica gel column chromatography yielded 10 fractions (III A-III J). All fractions were subjected to biological activity tests. The results of the rice growth inhibition bioassay and brine shrimp bioassay of all separated fractions indicated that fraction III C showed the highest activity both rice growth inhibition (59.98% for root length, 63.32% for leaf sheath at 10,000 ppm), and brine shrimp cytotoxicity test (LC_{50} 9.09 µg/ml). After reseparation the isolation, Compound 1 and Compound 2 were gained. Compound 1 is a white powder weight 41 mg, melting point 203-205 °C, and soluble in dichloromethane. Compound 2 is a white

powder melting point 168-170 °C, and soluble in dichloromethane. By means of spectroscopic identification (IR, NMR), Compound 1 was proved to be lupeol acetate while Compound 2 was triterpenoid with an acetyl group. Both isolated compound were retested the activity of the rice growth inhibition and brine shrimp bioassay .

The bioassay results of the isolated compounds showed in the opposited way for the rice growth. Pure compound of lupeol acetate did not show rice growth inhibition activity, but showed the promotion activity (sheath -23.00% for root length and -22.29% for leaves at 10,000 ppm). The result with brine shrimp bioassay experiment, pure compound of lupeol acetate showed medium activity (LC_{50} 40.37 μg/ml) and Compound 2 showed low activity with brine shrimp bioassay. In the initial fraction, fraction III showed highest activity for rice growth inhibition activity (100% both root length and leaf sheath). After separation, fraction III C was showed the highest activity with rice growth inhibition activity (59.98% for root length, 63.32% for leaf sheath at 10,000 ppm) and brine shrimp cytotoxicity test (LC_{50} 9.09 μg/ml), they were might be from the interaction of the components in this fraction, but after separation to the pure compound and test biological activity Compound 1 showed a medium activity with brine shrimp, and in the rice growth inhibition, it is the property of plant growth inhibitor that if we used it in a little quantity the plant growth inhibitor might became to plant growth promotor.

Compound 2 showed inhibition activity on rice (55.54%for root length and 39.64 % for leaf sheath at 10,000 ppm), and showed low activity (LC_{50} 101.57 μg/ml). From the result of rice growth inhibition activity it might concluded that Compound 2 was the active compound of this fraction.

However, this is the first report of brine shrimp bioassay properties of *E. heterophylla* Linn. The combined knowledge of the chemistry and biology would permit one to discover the useful compounds for specific purposes.