CHAPTER V

CONCLUSION

The immortalized mouse hippocampal cell line HT-22 cells, which resemble neuronal precursor cells were used as a model for studying the mechanism of oxidative glutamate toxicity. Glutamate exhibited its toxicity to the cells in a dose-dependent manner. HT-22 cells were killed by 50% of the control cells with glutamate at the concentration of 3.6 mM. The glutamate-induced toxicity involving in the oxidative damage by ROS could be attenuate by Trolox. Therefore, the glutamate-induced toxicity in the HT-22 cells is the suitable model for studying the neuroprotection of antioxidants.

In this study, various *P. mirifica* extracts, PMH, PME, PMM and PMW were first determined for their antioxidant activities by using the DPPH assay in order to screen for their potential to protect the HT-22 cells from the glutamate toxicity. Among these four *P. mirifica* extracts, only PMM and PMW appeared to exhibit the antioxidant activities against the DPPH radicals. It was likely that their antioxidant activities were the results of different compounds present in both extracts.

HPLC chromatograms of PMM and PMW showed distinctively different profiles. PMM contained high amount of genistein and daidzein compared with PMW. Both PMM and PMW inhibited the cell proliferation of HT-22 cells in certain degree. PMM was shown to be more vigorous in inhibition compared to PMW. However, PMM but not PMW exhibited the neuroprotection activity against oxidative stress from glutamate toxicity in HT-22 cells. PMM at the concentration of 50 and 100 μg/ml completely inhibited the toxicity of glutamate equivalent to 264 μg/ml of

Trolox. Genistein, and daidzein in the concentration ranges that cover the amount of daidzein and genistein found in 50 and 100 μg/ml of PMM did not shown any protection to cells against gluatamate toxicity.

The antioxidant activity of PMM against peroxyl radical by the ORAC assay were evaluated. PMM has much lower antioxidant activity against peroxyl radical than Trolox. Therefore, the direct scavenging property of PMM against peroxyl radical might not be the key protection mechanism of PMM against glutamate toxicity in HT-22 cells.

On the other hand, PMM exhibited excellent antioxidant activity against hydrogen peroxide. As H_2O_2 is converted into more reactive species, the most important of which is the hydroxyl radical. The neuroprotection mechanism of PMM, therefore, should be involved with the antioxidant activity against hydrogen peroxide or other radicals generated from hydrogen peroxide.

Because PMM is a mixture of genistein, daidzein, polyphenolic compounds and other substances, its protection could be resulting from combination of many compounds in the extract that mediate in different pathways to support each other (i.e. synergistic protection). Another interesting point is the concentration of PMM showing the protection against glutamate toxicity was found to inhibit cell proliferation. Therefore, PMM should be further purified and investigated for toxicity and protection activity of each pure substance in the extract.