CHAPTER V
CONCLUSIONS

1. The antioxidant activity of 95% ethanolic extract of 30 Thai medicinal plants was obtained from various plant parts. The plants were classified by family name. The results exhibited that that Euphorbiaceae family, which has an astringent taste, had a high level of antioxidant activity same the part used of fruit. Five plant extracts with the highest level of antioxidant activity, expressed as TEAC levels were *Phyllanthus emblica* Linn., *Terminalia chebula* Retz., *Morinda citrifolia* Linn., *Kaempferia parviflora* Wall. and *Houttuynia cordata* Thunb. respectively. In addition, the results show that *Phyllanthus emblica* Linn. contains the highest level of antioxidant activity, total phenolic and tannin content, antiglycation activity, and also lipid peroxidation inhibition activity. *Houttuynia cordata* Thunb. contains high levels of flavonoids. The correlation between total antioxidant activity and antiradical activity by TBARS was strongly positive. The present study suggests that these plants are a potential source of natural antioxidants which contain polyphenolic compounds such as rutin, gallic acid, pyrolgallol, catechin and caffeic acid as shown in the HPLC chromatogram. According to HPLC chromatogram, the major components of polyphenols of *Phyllanthus*

emblica Linn., *Terminalia chebula* Retz. and *Houttuynia cordata* Thunb. are rutin, gallic acid, pyrolgallol and catechin. *Morinda citrifolia* Linn. consist of rutin, gallic acid, pyrolgallol and caffeic acid. *Kaempferia parviflora* Wall. possibly consists of rutin.

2. *In vitro* study, the effects of antioxidant activity from 95% ethanolic extract of 30 Thai indigenous plants on diabetic oxidative stress was evaluated in different testing systems. The results show that in the model of lipid peroxidation, oxidative stress was generated in blood plasma of diabetes patients and resulted as the thiobarbituric acid-reactive substances (expressed as μM of MDA,). Plant extracts inhibited lipid peroxidation by their antioxidant activity. The results of co-incubation with plant extract at a concentration of 1 μg/ml, among the 30 plants, *Phyllanthus emblica* Linn. had the highest lipid peroxidation inhibition followed by *Lycopersicon esculentum* Mill., *Solanum torvum* Sw., *Houttuynia cordata* Thunb. and *Ocimum basilicum* Linn., respectively.

The protective effect of all plant extracts on glycation was determined based on inhibition activity per unit mass of glycation in μg/ml. Proteins (BSA) are modified by glucose creating advanced glycation end-products (AGEs). The free radicals have been shown to react in AGEs formation, which could be inhibited by using antioxidants. We found the protective effect of all plant extracts on glycation. *Phyllanthus emblica* Linn. had the highest level of inhibition of protein glycation followed by *Morinda*

citrifolia Linn., *Terminalia chebula* Retz., *Piper samentosum* Roxb. and *Artocarpus heterophyllus* Lamk.

3. The top five plants with the highest level of antioxidant activity were *Phyllanthus emblica* Linn., *Terminalia chebula* Retz., *Morinda citrifolia* Linn., *Kaempferia parviflora* Wall. and *Houttuynia cordata* Thunb. They were used as the raw materials to produce biologically fermented Thai indigenous plant beverage (BFPB). Their major components were analyzed using HPLC with the reference of standard polyphenolic compounds; rutin (R_T 3.29 min), gallic acid (R_T 5.56 min), pyrogallol (R_T 6.35 min), catechin (R_T 22.72 min) and caffeic acid (R_T 30.24 min). We found that the polyphenolic compounds were rich in the BFPB. These components were rutin, gallic acid, pyrolgallol and catechin. GC/MS profile shows that gallic acid (MW = 170.12, R_T 5.56 min), pyrolgallol (MW = 126.11, R_T 8.5 min) and one unidentified compound (R_T 4.5 min) were major constituents of BFPB. 1 ml of BFPB was found to have different levels of subclasses from polyphenolic compounds. Total phenolic content of 27.35 mg gallic acid equivalent, total flavonoid content of 2.24 mg quercetin equivalent and total tannins content of 0.05 mg tannic acid equivalent were found. An antioxidant activity index of 31.31 mg ascorbic acid equivalent/ml of BFPB was also evaluated.

4. In the *in vivo* study, the effects of BFPB could possibly involve free radical scavenging in diabetes. The administration of BFPB might reduce the physiological changes associated with diabetes, including normalized

energy utilization and metabolism. The effects of BFPB on oxidative stress in streptozotocin-induced diabetic rats were found as follows.

4.1 BFPB treatment at the doses of 2 and 6 ml/kg BW/day by oral gavage for 6 weeks to diabetic Wistar rat tended to increase body weight gain more than diabetic and untreated normal rats. Particularly, BFPB treatment at the dose of 2 ml/kg BW/day for 6 weeks increase body weight gain better than diabetic rats treated with 6 ml BFPB /kg BW/day.

4.2 Administration of BFPB at the doses of 6 ml/kg BW/day by oral gavage in diabetic rats for 6 weeks tended to reduce the increasing of plasma glucose when compared to rats which received 2 ml BFPB/kg BW/day and untreated rats. Interestingly, the change of plasma glucose levels in diabetic rats at the final experiment reduced the increase of plasma glucose by a dose dependent manner.

4.3 BFPB treatment at the doses of 2 and 6 ml/kg BW/day by oral gavage for 6 weeks to diabetic Wistar rat, showed a tendency to decrease lipid peroxidation between initiation to the 4^{th} week and the effect markedly reduced lipid peroxidation in diabetic rats at the 6^{th} week, in a dose dependent manner. Moreover, the dose of 6 ml/kg/day of BFPB obviously reduced MDA levels when compared with the diabetic group treated with 2 ml/kg BFPB throughout the experiment.

4.4 The doses of 2 and 6 ml BFPB/kg BW daily resulted in a decrease in the levels of erythrocyte ROS levels in diabetic rats during the

experiment but the effect was not different when compared with the untreated diabetic rat group. However, the decrease of erythrocyte ROS levels in each diabetic group clearly persisted until the end of the experiment when compared with the same group.

4.5 Administration of BFPB at dose of 2 ml/kg BW/day by oral gavage for 6 weeks to diabetic rats tended to reduce plasma superoxide anion and nitric oxide radicals, but the dose of 6 ml/kg BW/day tended to increase. The study implied that a high dose of BFPB would be pro-oxidant, promoting plasma superoxide and nitric oxide radicals which generation in diabetes. Since the half life of plasma superoxide anion and nitric oxide radicals are too short to measure, the effect of BFPB on them are uncertain, leading to underestimated values. It is essential that the concentration of both free radicals must be measured immediately.

The results of this study imply that a biologically fermented product from five Thai indigenous plants plays a role in improving glucose metabolism and reducing the oxidative stress in diabetes through scavenging free radicals inhibiting lipid peroxidation and inhibiting glycation. Biologically fermented Thai indigenous plant beverage may be a beneficial therapy, or at least delay the pathological conditions associated with oxidative stress, which lead to diabetic complications. On the basis of this study, these findings have led to the discovery and the evaluation of antioxidant molecules, polyphenolic compounds obtained from phytochemicals; flavonoids, phenols or tannins, such

as rutin, gallic acid, pyrolgallol, catechin and caffeic acid that synergistically inhibit, at an early stage of cell damage, the mechanism leading to diabetic complications. While waiting for new and other specific compounds, it is reasonable to suggest that the biologically fermented Thai indigenous plant beverage, which is an already-available substance, should be used because they are effective causal antioxidants. However, the use of BFPB should be further investigated for correlative and synergistic mechanisms in diabetes.